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THE ACTION OF NITRILES ON ORGANIC ACIDS.

BY CHARLES E. COLBY AND FRANK D. DODGE.

Two years since, in attempting to prepare some dibenzamide, we tried several methods, among which was one founded upon the reaction of Gautier,¹ by which he produced diacetamide on heating acetonitrile and acetic acid together in a sealed tube. Accordingly we heated equal molecules of benzonitrile and benzoic acid at 200° for four hours. Dibenzamide was formed, but the yield was small. It occurred to us that it might be well to apply the reaction to other nitriles and other acids, and we therefore experimented with a number of nitriles and acids and have obtained some remarkable results.

Gautier's experiments are described in full in the *Comptes Rendus*, 1868, Vol. 67, page 1255. According to his account, formonitrile and acetic acid produce acetamide; acetonitrile and acetic acid give diacetamide; and propionitrile and acetic acid give a mixture of triacetodiamide and diacetopropionamide. We have thought it worth while to repeat the last two of these experiments, and our results were in one case the same as, and in the other case different from, Gautier's.

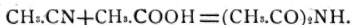
Before describing our experiments in detail, it is well to state that we first made sure of the purity of our materials. The nitriles were freshly distilled over phosphorus pentoxide, and the acids were ascertained to be free from water. Equal molecules of nitrile

¹ *Ztschr. Chem.*, 1869, 127.

and acid were heated in a sealed tube. In several cases from three to five drops of acetic anhydride were also added to the tube, and we think the yield of product was thereby increased. After heating, a diminished pressure was observed in every case on opening the tube. The contents of the tubes were generally evaporated to dryness on a water-bath, when the uncombined nitrile or acid present was easily volatile; or were washed with dilute sodium carbonate solution, when aromatic acids were to be removed. Uncombined aromatic nitriles were recovered by distillation with steam or by extraction with ether. The residue, or insoluble non-volatile portion, was then crystallised from alcohol or ether. We submit a few of our experiments in detail.

1. *Acetonitrile on Acetic Acid.*

This is a repetition of one of Gautier's experiments. We heated 8.2 grams of acetonitrile and 12 grams of acetic acid in a sealed tube at 250° for seven hours. On opening the tube no pressure was noticed. Instead of distilling the contents of the tube, as Gautier did, we found it better to evaporate to dryness in a dish on the water-bath. A solid residue remained. This dissolved in ether, and, left to itself, the solution deposited white crystals, which were recognised as the diacetamide (m. p. 82°) described by Strecker¹ and Gautier. The reaction may be written thus:

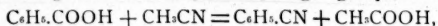


2. *Acetonitrile on Benzoic Acid.*

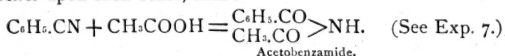
Five tubes were filled each with a mixture of 4 grams of acetonitrile and 12 grams of benzoic acid. They were all heated at 150°–160° for three hours. One tube was then opened and the solid crystalline contents furnished nearly the original quantity of benzoic acid. The four remaining tubes were further heated as follows: After five hours at 175° and five hours at 200° they appeared to be unchanged, but after heating five hours longer at 220° the contents of the tubes had changed color and apparently consisted of white crystals wet with a brown liquid. A second tube was then opened. The crystals all dissolved in dilute sodium carbonate and proved to be unaltered benzoic acid. Acetic acid was also recognised here. The brown liquid was extracted with

¹Ann. Chem. (Liebig) 103, 327.

ether, which on evaporation left a residue of benzonitrile to the extent of 3 grams. The three remaining tubes were now heated for six hours at 220°–230°, for seven hours at 230°–240°, and for eight hours at 250°–260°. The contents had by this time become completely liquid. A third tube was then opened, and from it we obtained a smaller quantity of benzoic acid, a larger quantity of acetic acid, and 7 grams of benzonitrile. From a fourth tube opened about this time we obtained, by cautious evaporation of the benzonitrile extracted from it, a very small quantity of acetobenzamide, $C_6H_5.CO.NH.CO.CH_3$, which will be described later. The fifth and last tube was further heated for seven hours at 280°. The contents, extracted in the same way, gave the same quantities of the same products as the third tube. The reaction has therefore been different from what we expected. The two alcohol residues have exchanged their carboxyl and cyanogen groups, thus:

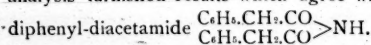


and at a temperature of 260° the yield of benzonitrile has amounted to 70 per cent. of the theoretical quantity. The production of acetobenzamide in the fourth tube is explained by supposing that the benzonitrile and acetic acid first formed react further upon each other, thus:



3. Acetonitrile on Phenyl-acetic Acid.

4 grams of acetonitrile and 13.6 grams of phenyl-acetic acid were heated for twenty hours at 250°. The tube was rinsed out with weak sodium carbonate solution and the residue dissolved in ether. The ether on evaporation furnished a small quantity of benzyl cyanide, besides a crystalline residue. This latter was washed with cold alcohol and recrystallised from hot alcohol. We thus obtained long white needles, melting at 192°. An elementary analysis furnished results which agree with the composition of

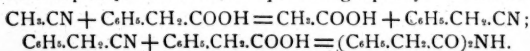


I. 0.2010 gram substance gave 0.1111 gram H_2O and 0.5587 gram CO_2 .

II. 0.2263 gram substance gave 11.5 cc. nitrogen, measured at 26° and 766 mm.

	Found.		Calculated for $C_{16}H_{15}NO_2$.
	I.	II.	
C	75.80	...	75.87
H	6.14	...	5.96
N	...	5.68	5.53
O	...	...	12.64

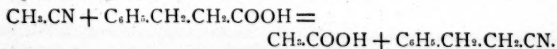
The composition of this body was further proved by saponifying it with sodium hydrate, when it gave only phenyl-acetic acid and ammonia. In the alkaline water used for rinsing the tube we recognised acetic and phenyl-acetic acids. Therefore in this experiment the cyanogen and carboxyl groups have in part exchanged places and the resulting benzyl cyanide has combined with some of the unaltered phenyl-acetic acid, thus producing diphenyl-diacetamide:



4. Acetonitrile on Phenyl-propionic Acid.

4 grams of nitrile and 15 grams of acid were heated for twenty hours at 250° . The product of the reaction was a dark brown oil. This was agitated with soda solution to remove unaltered phenyl-propionic acid, and then distilled with steam. A nearly colorless oil passed over, which was recognised by its odor and by its boiling point (255°) as phenyl-propionitrile. It was completely saponified by sodium hydrate, yielding 2 grams of phenyl-propionic acid.

The conversion of phenyl-propionic acid into the corresponding nitrile under the influence of acetonitrile was therefore in this case only partial. But in another trial, where we used 8. grams of acetonitrile and 15 grams of phenyl-propionic acid, we obtained a yield of nearly 13 grams of phenyl-propionitrile. We have in fact used this method to prepare a considerable quantity of phenyl-propionitrile for further experiments. None of our experiments under this head have yielded a secondary amide. The following equation expresses the reaction:



5. Propionitrile on Acetic Acid.

Equal molecules of these substances, with three drops of acetic anhydride, were heated in a sealed tube for seven hours at 250° .

The contents of the tube were then evaporated to dryness in a dish on a water-bath. A solid residue remained which was crystallised from ether. We thus obtained white needles which had a melting point of 86° .

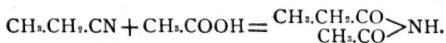
This is really a repetition of another of Gautier's experiments. By the reaction of the same materials he obtained a compound which he called diacetopropiondiamide (m. p. 68°). Our crystals (m. p. 86°), which were isolated by a method different from his, furnished on analysis the following results:

I. 0.0463 gram substance gave 0.03305 gram H_2O and 0.0885 gram CO_2 .

II. 0.1018 gram substance gave 11.6 cc. nitrogen, measured at 26° and 761 mm.

	Found.		Calculated for Diacetopropiondiamide, $(CH_3CO)_2NH + CH_3CH_2.CO.NH_2$.	Calculated for Acetopropionamide, $CH_3CH_2.CO$ $CH_3CO > NH$.
C	I. 52.13	II. ...	48.28	52.17
H	7.93	...	8.05	7.83
N	...	12.29	16.09	12.17
O	...	...	27.58	27.83

Our compound therefore corresponds in composition more nearly to acetopropionamide than to Gautier's product. The small yield which we obtained has precluded any further experiments upon it. The reaction may be written thus:



6. Propionitrile on Propionic Acid.

11 grams of nitrile and 15 grams of acid, with three drops of acetic anhydride, were heated at 250° for seven hours. The product of the reaction was a dark-colored liquid containing long needle-like crystals. The whole contents of the tube were evaporated to dryness on a water-bath and the solid residue crystallised from ether. Long white needles were formed, melting at 152° , which are probably the dipropionamide. Analysis and investigation of this substance were discontinued in consequence of the appearance of an article by Otto and Tröger¹ describing the formation and properties of dipropionamide.

¹ Ber. d. chem. Ges. 23, 759.

7. *Benzonitrile on Acetic Acid.*

30 grams of nitrile, eighteen grams of acid and three drops of acetic anhydride were heated in a sealed tube at 220° for five hours. The contents of the tube were evaporated to dryness on a water-bath and the residue crystallised from alcohol. White crystals were obtained which had a melting point of 115° . The yield was significant. Analysis of these crystals furnished the following figures:

I. 0.2145 gram substance gave 0.1116 gram H_2O and 0.5201 gram CO_2 .

II. 0.2009 gram substance gave 0.1033 gram H_2O and 0.4878 gram CO_2 .

III. 0.2050 gram substance gave 16 cc. nitrogen, measured at 20° and 760 mm.

	I.	Found. II.	III.	Calculated for $C_6H_5.CO.NH.CO.CH_3$
C	66.13	66.22	...	66.26
H	5.78	5.71	...	5.52
N	...	...	8.71	8.59
O	...	...	...	19.63

Heated with alcoholic potassium hydrate, the substance yields ammonia, acetic acid and benzoic acid, and we think, therefore, that it is acetobenzamide, $C_6H_5.CO.NH.CO.CH_3$. It is readily soluble in hot water, in alcohol and in benzene. Addition of sodium to the solution in benzene causes evolution of hydrogen and precipitation of the sodium compound. We have prepared the silver compound also.

The reaction which has produced acetobenzamide is probably $C_6H_5CN + CH_3.CO.OH = C_6H_5.CO.NH.CO.CH_3$. (Compare Exp. 2.)

8. *Benzonitrile on Phenyl-acetic Acid.*

10 grams of nitrile and 13.6 grams of acid, with three drops of acetic anhydride, were heated for five hours at 200° , then for five hours at 240° , and finally for two hours at 260° . We obtained here a solid product which was at once crystallised from hot alcohol. White crystals were isolated having a melting point of 171° . The yield was good. Analysis shows that they are probably phenyl-acetobenzamide.

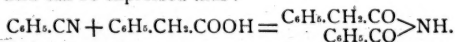
I. 0.2000 gram substance gave 0.1043 gram H_2O and 0.5521 gram CO_2 .

II. 0.1985 gram substance gave 10.2 cc. nitrogen, measured at 26° and 775 mm.

	I.	Found.	II.	Calculated for $\text{C}_6\text{H}_5\cdot\text{CH}_2\cdot\text{CO}>\text{NH}$.
C	75.28	...	75.31	
H	5.79	...	5.44	
N	...	5.82	5.85	
O	...	...	13.39	

Treated with two molecules of sodium hydrate in alcoholic solution the substance gave ammonia and a mixture of benzoic acid and phenyl-acetic acid.

It is evident that the reaction between benzonitrile and phenyl-acetic acid can be expressed thus :

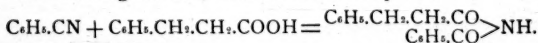


9. Benzonitrile on Phenyl-propionic Acid.

5 grams of nitrile and 7 grams of acid, with five drops of acetic anhydride, were heated for five hours at 200°, five hours at 240°, and two hours further at 260°. The product, purified in the usual way, furnished a good yield of transparent white needles when crystallised from alcohol. They melted at 106°. On saponification with sodium hydrate they gave off ammonia and yielded benzoic acid and phenyl-propionic acid. The ultimate analysis gave results agreeing with the composition of phenyl-propionbenzamide, $\text{C}_6\text{H}_5\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CO}\cdot\text{NH}\cdot\text{CO}\cdot\text{C}_6\text{H}_5$.

	I.	Found.	II.	Calculated for $\text{C}_{18}\text{H}_{19}\text{NO}_2$.
C	75.71	...	75.89	
H	6.06	...	5.93	
N	...	6.06	5.53	
O	...	...	12.65	

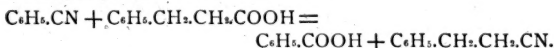
The following reaction, therefore, has taken place :



Another tube was filled with double the above quantities of nitrile and acid and heated for twenty hours at 280°. The product was purified as usual and furnished a very small amount of the phenyl-propionbenzamide ; but 7 grams of benzoic acid were obtained from the alkaline wash-water, and nearly 10 grams of

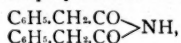
phenyl-propionitrile, $\text{C}_6\text{H}_5\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CN}$, boiling from 253° to 256° , were furnished by the ether extraction. The fact that phenyl-propionitrile was formed was further proved by converting it into phenyl-propionic acid by saponification with sodium hydrate.

The longer-continued heating at a higher temperature has in this case produced a different result from that obtained in the case of the first tube. The cyanogen and carboxyl groups have exchanged places, thus:



10. *Phenyl-acetonitrile (Benzyl Cyanide) on Acetic Acid.*

Equal molecules of these materials were heated at 260° for six hours. We obtained in this case a mass of white crystals, which were washed with cold alcohol, to remove unaltered nitrile and acid, and then crystallised from hot alcohol. The purified substance formed white needle crystals melting at 192° . It corresponds in appearance and properties to the diphenyl-diacetamide,



described in Exp. 3. A nitrogen determination gave a result agreeing with the results of analysis of that substance. The yield was small. We account for the formation of diphenyl-diacetamide in this experiment by supposing that a part of the phenyl-acetonitrile became converted into phenyl-acetic acid, and that this then combined with the remaining phenyl-acetonitrile to form a simple secondary amide. If this were so we ought to find acetonitrile and phenyl-acetic acid as by-products, but we were unable to detect either of them.

11. *Phenyl-acetonitrile on Benzoic Acid.*

Equal molecules of nitrile and acid were heated for five hours at 240° , and then two hours at 260° . The product, isolated as in the preceding experiment, formed, when crystallised from alcohol, long white needles whose melting point was 171° . The yield was good and no benzonitrile was found as by-product. The acids obtained on saponification and the figures furnished by elementary analysis lead us to the conclusion that this is the same phenyl-acetobenzamide produced in Exp. 8.

	Found.	Calculated for $\text{C}_6\text{H}_5\cdot\text{CH}_2\cdot\text{CO}>\text{NH}\cdot$ $\text{C}_6\text{H}_5\cdot\text{CO}$
C	74.91	75.31
H	5.86	5.44
N	5.35	5.85
O	—	13.39

The reaction is therefore parallel with that in Exp. 8,



12. Phenyl-acetonitrile on Phenyl-acetic Acid.

16 grams of nitrile and 19 grams of acid were heated at 250° for ten hours. After washing out the excess of phenyl-acetic acid by dilute soda solution and rinsing the residue with cold alcohol to remove any unaltered phenyl-acetonitrile, there remained a crystalline mass which, dissolved in hot alcohol and left to cool, deposited the same white needle crystals of diphenyl-diacetamide (m. p. 192°) obtained in Exps. 3 and 10. The yield was unusually good. The following figures were obtained on analysis:

I. 0.2005 gram substance gave 0.1076 gram H_2O and 0.5544 gram CO_2 .

II. 0.2059 gram substance gave 10.4 cc. nitrogen, measured at 22° and 767 mm.

	I.	Found.	II.	Calculated for $\text{C}_6\text{H}_5\cdot\text{CH}_2\cdot\text{CO}>\text{NH}\cdot$ $\text{C}_6\text{H}_5\cdot\text{CH}_2\cdot\text{CO}>\text{NH}\cdot$
C	75.41	...	...	75.87
H	5.96	...	...	5.96
N	...	5.77	...	5.53
O	...	...	...	12.64

When saponified by alcoholic sodium hydrate the diphenyl-diacetamide yielded ammonia and the theoretical quantity of phenyl-acetic acid. It is therefore a secondary diacid amide. The reaction by which it is formed may be thus represented:



Diphenyl-diacetamide is soluble with difficulty in cold alcohol but dissolves readily in hot. It is slightly soluble in hot water.

We have tried other nitriles and acids than those mentioned and have made some fifty experiments altogether. We select twenty of them, including the twelve foregoing, and place our results in a tabular form.

PRODUCTS.

MATERIALS.

MATERIALS.				PRODUCTS.			
NITRILE.	ACID.	TEMP.	HOURS.	NITRILE.		ACID.	NAME.
				NAME.	d ₄ °		FORMULA.
Acetonitrile.....	Acetic acid.....	250°	7		...	Acetic acid.....	Diacetamide.....
".....	Benzoic acid.....	260	4	Benzonitrile.....	192°	".....	Aceto-benzamide.....
".....	Phenyl-acetic acid.....	250	20	Phenyl-acetonitrile.....	232	".....	Diphenyl-diacetamide.....
".....	Phenyl-propionic acid.....	250	20	Phenyl-propionitrile.....	255	".....	(C ₆ H ₅ .CH ₂ .CO) ₂ NH.....
Propionitrile.....	Acetic acid.....	250	7		...		Aceto-propionamide.....
".....	Propionic acid.....	250	7		...		Dipropionamide.....
".....	Ortho-toluic acid.....	235	5	Ortho-toluic nitrile.....	204	Propionic acid.....	(CH ₃ .CH ₂ .CO.NH.CO.CH ₃).....
".....	Meta-toluic acid.....	235	5	Meta-toluic nitrile.....	212	".....	(CH ₃ .CH ₂ .CO) ₂ NH.....
".....	Cinnamic acid.....	235	5	Cinnamic nitrile.....	257	".....	
Benzonitrile.....	Acetic acid.....	220	5		...		Aceto-benzamide.....
".....	Benzoic acid.....	260	4		...		Dibenzamide.....
".....	Phenyl-acetic acid.....	250	7		...		Phenyl-acetobenzamide.....
".....	Phenyl-propionic acid.....	250	7		...		Phenyl-propionbenzamide.....
".....	".....	280	20	Phenyl-propionitrile.....	255	Benzoic acid.....	
Phenyl-acetonitrile.....	Acetic acid.....	260	6		...		Diphenyl-diacetamide.....
".....	Propionic acid.....	230	12		...		Phenyl-acetopropionamide.....
".....	Butyric acid.....	230	12		...		C ₆ H ₅ .CO.NH.CO.CH ₂ .C ₆ H ₅
".....	Benzoic acid.....	250	7		...		C ₆ H ₅ .CO.NH.CO.CH ₂ .C ₆ H ₅
".....	Phenyl-acetic acid.....	250	10		...		Phenyl-acetobenzamide.....
".....	Phenyl-propionic acid.....	250	7		...		Diphenyl-diacetamide.....
".....					...		(C ₆ H ₅ .CH ₂ .CO) ₂ NH.....
".....					...		Phenyl-aceto-phenyl-propionamide.....
".....					...		C ₆ H ₅ .C ₆ H ₄ .CO > NH.....
".....					...		C ₆ H ₅ .C ₆ H ₅ .CO.....

This table shows on the left hand the nitriles and acids selected, with the number of hours and the temperatures at which they were heated. The products which we have thus far isolated and identified—nitrile, acid or secondary amide—are given in the proper right-hand columns. In cases where an amide was formed, other columns show its formula and melting point. These amides are all soluble in hot alcohol and most of them crystallise on cooling. Saponification with the calculated quantity of sodium hydrate gave in each case ammonia and the appropriate acids. In every case where a nitrile was one of the products it was identified by its boiling point and also by converting it into the corresponding acid.

The experiments with propionitrile on ortho-toluic, meta-toluic and cinnamic acids, and with phenyl-acetonitrile on propionic, butyric and phenyl-propionic acids, need no special description.

The examples in this table were not selected with the idea of advancing any particular theory; they embrace all the experiments made with these four nitriles. In a future article we shall give the results obtained with other nitriles. The reactions here involved may be summarised into three groups:

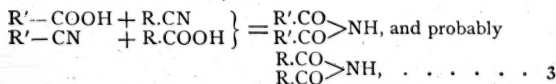
The nitrile and acid have condensed into a secondary, or diacid, amide, thus:



The alcohol radicles have exchanged their cyanogen and carboxyl groups:



In two instances the latter reaction was evidently accompanied by a tendency on the part of the products to condense with some of the unaltered materials, thus:

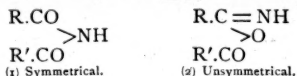


So far as these experiments indicate, we note that:

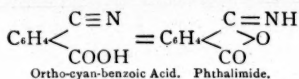
1. Fatty nitriles and fatty acids have given secondary amides.
2. Fatty nitriles and aromatic acids have exchanged their cyanogen and carboxyl groups, giving fatty acids and aromatic nitriles.
3. Aromatic nitriles and fatty acids have given mixed secondary amides.

4. Aromatic nitriles and aromatic acids have given secondary amides, with the exception of one instance where the temperature was unusually high. In that case the product was a nitrile of the higher radicle.

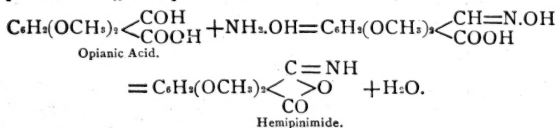
There are two possible constitutional formulæ for these secondary amides:



The first is the one generally accepted. An argument in favor of the second formula is, that ortho-cyan-benzoic acid, obtained from ortho-amido-benzoic acid by Sandmeyer's¹ reaction, on standing changes into phthalimide. This change is entirely analogous to the reaction which has produced the above-described secondary amides, and Liebermann² explains it thus:



Several reasons exist for considering this formula to represent the structure of phthalimide. Liebermann gives one reason by showing that opianic acid reacts with hydroxylamine, the final product being hemipinimide:



This reaction seems to be more easily explained by the unsymmetrical formula.

Further experiments will be necessary to determine which constitutional formula is to be assigned to the secondary amides described in this paper.

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¹ Ber. d. chem. Ges. 18, 1496.

² Ibid. 19, 2281.

Contributions from the Chemical Laboratory of the Department of Agriculture.

XVI.—ON THE METHODS OF ESTIMATION OF THE FATTY BODIES IN VEGETABLE ORGANISMS.

BY W. MAXWELL.

These investigations have been conducted with the purpose of acquiring some further data showing the comparative proportions of matters which become separated from vegetable organisms under treatment with pure ether in varying durations of time. It has likewise been attempted to determine whether the whole of the substituted glycerides, or lecithines, may be separated by direct extraction with ether alone.

The following experiments were made with the seeds of *Gossypium* (cotton seed), the materials being reduced to the greatest state of fineness and the extractions made with the "Knorr" extraction apparatus. The calculations are reduced to the standard of water-free material.

The preliminary analyses which were made in order to establish the proportions of the vegetable matters separated by the action of pure ether in different durations of time, gave the following results :

Time.	Weight of material. Grams.	Weight of extract. Grams.	Mean of 4 analyses. Per cent.
8½ hours,	4.0	1.472	37.39
10 "	4.0	1.491	37.87
15 "	4.0	1.511	38.38

These results, although based upon the mean of four analyses, were accepted only as following a number of tests previously made as preparatory observations.

It will be observed that an increased quantity of substance is separated with an extension of the period of extraction. The proportion of the increase is, however, small; and it is not conclusive as showing that the greater duration of time causes an increase in the fatty bodies only, as other constituents of vegetable organisms can be affected by a very protracted treatment with ether. In consequence of these reasons it has been resolved to estimate the amounts of the fatty bodies separated by extraction with pure ether during a period of 15 hours.

In considering this estimation of the proportions of glycerides and substituted glycerides or lecithines which are contained in the ether extract, the presence of other bodies, which have been removed by the ether, makes it impossible, in the absence of an absolute analysis of the extract, to determine accurately the quantitative relations of those fatty bodies. The ether extracts of plant matters contain in most instances varying amounts of the cholesterolins, and, in some instances, of solid hydrocarbons, and these bodies may constitute several per cent. of the extract, as has been shown by E. Schulze¹ and others. Although the precise percentage of the normal glycerides cannot be estimated without an absolute analysis of the total ether extract, the substituted glycerides, or lecithines, may be determined with absolute accuracy, as the estimation of these bodies is based upon the proportion of phosphorus contained in the ether extract. The extract is burned with mixed alkali salts, by which process the phosphorus present in the organic form as lecithine becomes fixed as an alkali phosphate. The phosphorus is determined in the usual way as magnesium pyrophosphate, and the weight of the latter body, multiplied by the accepted Hoppe-Seyler factor, gives the amount of lecithine.

A further series of extractions of the finely powdered cotton seed material was made with pure ether for a period of 15 hours each and the total extract determined. The proportion of lecithines contained in the extract was determined by the method already given, the residue of the extract being considered as composed of glycerides and other bodies. The means given by eight analyses were as follows:

Material. Grams.	Extract. Grams.	Mg. pyrophosphate. Grams.	L. in extract. Per cent.	L. in seed. Per cent.
4.0	1.513	0.00225	1.08	0.409

It will be shown by the following investigations that the proportion of substituted glycerides, or lecithines, stated (0.409 per cent.) does not represent the total lecithines present in the cotton seed.

The observation was made simultaneously by Prof. E. Schulze at Zürich and myself at Harvard College, that the lecithines are not wholly separated by treatment of the vegetable material with ether even for a long period of time. Prof. Schulze² observed

¹ Ztschr. physiol. Chem. 14, 491.

² Ibid. 13, 365.

that the results obtained by me in determinations of the amounts of lecithines contained in given seeds were too low, as the following numbers indicate:

	<i>Vicia sativa.</i>	<i>Faba vulgaris.</i>
W. Maxwell	= 0.55 per cent.	0.60 per cent.
E. Schulze	= 1.22	0.81

At the same time that Prof. Schulze was making the above observations with the seeds of the vetch and horse-bean, I, in experiments made with *Phaseolus vulgaris*, arrived at the same conclusion, viz., that the lecithines are not capable of a complete separation when the material is treated with ether alone. It was found that if the material which had been treated with ether were further extracted with absolute alcohol, a second portion of lecithines was obtained, which, in most instances, was greater than the proportion separated by the ether. The material, which had already been extracted for 15 hours with ether, was extracted for 6 hours with absolute alcohol. The alcoholic extract was evaporated to dryness, and the residue immediately re-extracted with ether. The reason for taking up the lecithines out of the alcohol extraction residue was, in the first place, on account of the observation that those lecithines which were originally insoluble in ether are capable of being dissolved by ether after they have been separated from the vegetable material by the treatment with alcohol; and further, the re-extraction with ether was made in order to avoid any possible small portion of inorganic phosphates which might have been removed from the plant material by the treatment with alcohol. It was found, however, that only a mere trace of mineral phosphates had been separated by the action of the alcohol. The following numbers indicate the proportions of fatty bodies separated by extraction with ether alone, and with ether, followed by the alcohol-ether, as described already, and the amounts of lecithines contained in the respective extracts:

Solvent.	Material. Grams.	Extract. Grams.	Mg ₃ P ₂ O ₇ . Grams.	L. in extract. Per cent.	L. in seed. Per cent.
Ether	4.0	1.513	0.00225	1.08	0.409
Ether + alcohol-ether	4.0	1.603	0.00516	2.306	0.94

The results given, as obtained by the successive treatment with ether and alcohol-ether, are the means of eight analyses.

The experiments have shown that not only are the lecithines not wholly separated by treatment with ether, but that even the

greater portion of these bodies resists the action of the ether, and may be separated only by the succeeding treatment with alcohol-ether. In the example of the cotton seed, the proportion of lecithines in the total fatty bodies is very small, yet it has been shown that more than 50 per cent. of the substituted glycerides are left in the material after extraction with ether for a period of 15 hours. If certain other varieties of seeds be considered, such as the legumes quoted in a previous place, it has been found that the total ether and alcohol-ether extracts of those seeds—which seeds contain from 2.5 per cent. to 4 per cent. respectively of fatty bodies—are composed of proportions of lecithines varying from 25-45 per cent. If, as has been shown, as much as 50 per cent. of the lecithines may remain unseparated after treatment of the materials with ether only, it is thus apparent that, in the examples of seeds in which the fatty bodies are composed of the large proportions of lecithines stated, from 20-25 per cent. of these fatty bodies remain undetermined in all estimations where ether only is used.

XVII.—ON THE BEHAVIOR OF THE FATTY BODIES,
AND THE ROLE OF THE LECITHINES,
DURING NORMAL GERMINATION.

BY W. MAXWELL.

In a preceding article, treating of "Methods of Estimation of the Fatty Bodies in Vegetable Organisms," the author has shown the dissimilar behavior of the glycerides and lecithines under the action of given solvents. It has been observed that when certain vegetable materials are treated with pure ether for a given period of time, the normal glycerides are wholly dissolved; but, on the other hand, only a part, and in some instances less than 50 per cent., of the lecithines are directly acted upon by the ether; and further, that only under the action of absolute alcohol can the residue of these substituted glycerides, which are left in the material after extraction with ether, be entirely separated.

The study of the respective glycerides under the action of artificial solvents, though suggestive of, was preliminary to, the investigation of these bodies, and of the lecithines especially, under the influences of normal germination.

The presence of bodies in vegetable organisms, such as the cholesterins, hydrocarbons, etc., which may be separated from the material with the fatty bodies by treatment with ether and alcohol, makes it impracticable to attain definite results in respect of the normal glycerides without an absolute analysis of the total extract, *i. e.*, without saponifying the extracts and determining severally the saponification products. Moreover, the germinated materials yield still greater quantities of non-fatty bodies to the action of ether and alcohol, and the difficulty of the estimation of the normal glycerides in the mature seed thus becomes aggravated in the germinated materials. The pursuit of the lecithines in the products of germination may be made on the same principle as is the estimation of these bodies in the mature seed, which rests upon the determination of the organic phosphorus separated from the materials with ether and alcohol-ether as magnesium pyrophosphate and the calculation of the lecithines from that compound by means of the Hoppe-Seyler factor.

The following investigations will indicate the disposition of the fatty bodies in general under the action of germination. The specific purpose, however, has been the observation of the lecithines during the initial stages of growth, and under the influences of the ferments of the vegetable organism.

The preparation of the germinated seed-materials was as follows: The seeds of *Phaseolus vulgaris* were prepared in the propagating house of the Botanical Garden connected with Harvard College, and under the direction of Professor Goodale. The other varieties were germinated in the propagating house of the Department of Agriculture, Washington. The seeds were planted under the conditions of light, temperature and moisture as approximately normal as those of a propagating house can be made. The growth was stopped in the several stages at specific terms of development, and not at the end of specified periods of time, as is usual. The checking of the growth was done by placing the materials in an oven at a temperature of 90° C. The stages of growth at which the germination was stopped were marked by the following indications of development:

1st Stage.—The radicle had protruded through the testa about three-fourths of an inch. After drying, the seed and radicle together were ground to a fine powder.

2d Stage.—The plumule was developed and the radicle proportionally increased.

3d Stage.—The reserve materials of the mother-seed had become wholly used up, nothing but the testa remaining. So that in the last stage the plantlet itself formed the material, and at that period of its development when dependence on the outer world was attained.

The study of the seeds of *Phaseolus vulgaris* was conducted in the laboratory of Harvard College in 1889. The results obtained with this variety will be given in detail and first in order. The work upon the other varieties, cotton seed and maize, was done later in the laboratory of the Department of Agriculture.

Phaseolus vulgaris (mature seed).

Material.	Extract.	Per cent.
(1). 5.006 grams.	0.161 gram.	3.216
(2). 5.872	0.184	3.133
		Mean = 3.174

The proportion of lecithines contained in 100 parts of the extract and 100 parts of the material was found to be as follows:

Material. Grams.	Extract. Grams.	Mg ₃ P ₂ O ₇ . Grams.	L. in material. Per cent.	L. in extract. Per cent.
10.877	0.345	0.01397	0.933	29.394

No. 1 Stage Germination.

Material.	Extract.	Per cent.
(1). 10.13 grams.	0.2725 gram.	2.69
(2). 6.827	0.1960	2.871
		Mean = 2.78

The percentages of lecithines in the extract and in material were as follows:

Material. Grams.	Extract. Grams.	Mg. phosphate. Grams.	L. in material. Per cent.	L. in extract. Per cent.
(1). 10.13	0.2725	0.0235	1.934	61.945
(2). 6.827	0.1960	0.0165	1.748	60.91
			Means = 1.841	61.427

No. 2 Stage Germination.

In this stage the mother-seed was wholly exhausted and the material of the experiment was composed of the young plantlet. The plantlet was divided into two sections by cutting its axis midway between the extremities, giving thus the radicle and the

plumule sections. The purpose of this division of the plantlet into radicle and plumule sections was in order to study these parts separately, and thus to be able to observe the distribution of the lecithines, if these bodies were present, in the general organism of the plantlet.

(a). Radicle section :

Material.	Extract.	Per cent.
(1). 5.5 grams.	0.2215 gram.	4.03
(2). 4.922	0.1865	3.79
Mean = 3.909		

The percentages of lecithines in the extract and in the material :

Material. Grams.	Extract. Gram.	Mg ₃ P ₂ O ₇ . Gram.	L. in material. Per cent.	L. in extract. Per cent.
(1). 5.5	0.2215	0.03	3.96	98.41
(2). 4.922	0.1865	0.025	3.48	91.90
Means = 3.72				95.15

(b). Plumule section :

Material.	Extract.	Per cent.
(1). 9.633 grams.	0.293 gram.	3.042
(2). 8.929	0.258	2.880
Mean = 2.961		

The percentages of lecithines in the extract and in the material :

Material. Grams.	Extract. Gram.	Mg ₃ P ₂ O ₇ . Gram.	L. in material. Per cent.	L. in extract. Per cent.
(1). 9.633	0.293	0.038	2.86	94.26
(2). 8.929	0.258	0.0315	2.63	88.00
Means = 2.75				91.13

Mean of radicle and plumule = 3.23 per cent.

The investigation of the two stages of germinated materials shows that the first-stage material contained twice the proportion, and the second stage three times the proportion, of lecithines which was found in the original or mature seed. The study of the two sections of the plantlet in the second stage indicates that the lecithines were equally distributed through the whole organism. The conclusions indicated by these results will be considered later.

Gossypium (Cotton seed).

The results given in each number of the following series represent the mean of four determinations :

Material.	Extract.	Per cent.
Mature seed, 1 gram.	0.4076 gram.	40.764
No. 1 stage germ. 1 "	0.3022 "	30.220
" 2 " " 1 "	0.1797 "	17.970
" 3 " " 1 "	0.1082 "	10.820

Percentages of lecithines in the material and in the extract :¹

Material.	Extract. Gram.	Mg ₃ P ₂ O ₇ . Gram.	L. in material. Per cent.	L. in extract. Per cent.
Mature seed, 1 gram,	0.4076	0.001293	0.94	2.306
No. 1 stage, 1 "	0.3022	0.00296	2.15	7.110
" 2 " 1 "	0.1797	0.00274	2.00	11.080
" 3 " 1 "	0.1082	0.00180	1.308	12.00

It is shown that the first-stage germination material contained nearly three times the proportion of lecithines which was present in the mature seed; but the second, and, still more, the third stages indicated that the high proportion of those bodies which appears in the first stage had become in part reduced.

Zea mays (Maize).

In the work done with the maize variety large amounts of material were taken, it having been observed in the preliminary experiments that the proportion of lecithines contained in the corn is very small.

Material.	Extract.	Per cent.
Mature seed, 16 grams.	0.9122 grams.	5.70
No. 1 stage, 18 "	0.9169 "	5.09
" 2 " 18 "	1.0634 "	5.90
" 3 " 14 "	1.0374 "	7.41

Percentages of lecithines in the material and in the extract :

Material.	Extract. Gram.	Mg ₃ P ₂ O ₇ . Gram.	L. in material. Per cent.	L. in extract. Per cent.
Mature seed, 16 grams.	0.9122	0.0041	0.186	3.300
No. 1 stage, 18 "	0.9169	0.0048	0.193	3.800
" 2 " 18 "	1.0634	0.0079	0.319	5.400
" 3 " 14 "	1.0374	0.0084	0.436	5.886

A comparison of the proportions of lecithines contained in the mature seeds, and in the materials of the several stages of germination of the three varieties of seeds under investigation, is shown as follows :

¹ The analytical work in these examples was made by Mr. Omar Carr.

	<i>Phaseolus vulgaris.</i>	<i>Gossypium.</i>	<i>Zea mays.</i>
Mature seed,	0.933 per cent.	0.94 per cent.	0.186 per cent.
No. 1 stage,	1.841 "	2.15 "	0.193 "
" 2 "	3.230 "	2.00 "	0.319 "
" 3 "	...	1.308 "	0.436 "

The estimation of the proportions of the lecithines contained in the germination materials of the varieties of seeds specified has been based on the principle that the organic phosphorus found in the ether and alcohol-ether extracts of these materials is present in the form of lecithine. It is not, however, conclusive that the phosphorus separated by the given solvents was in combination with a glyceride. In order to have a proof that the phosphorus compound separated from the material of the germinated seeds was a lecithine, it was necessary to examine the decomposition-products of the latter body, which was supposed to be contained in the extracts of the materials. For this purpose was taken the No. 3 stage germination-material of the cotton seed, in which the mature seed had been wholly used up and the material consisted wholly of the young plantlet. About 30 grams of this material were extracted, and, after evaporating off the ether and alcohol, the extract-residue was boiled for about half an hour with barium hydroxide. After removal of the excess of barium and partial evaporation of the solution, a very large quantity of barium stearate separated out in a well-crystallised form. After the separation of the barium stearate salts the solution was evaporated to a thick syrup, which was extracted with 99 per cent. alcohol containing 1.5 per cent. hydrochloric acid. If cholin, the most characteristic of the decomposition-products of a lecithine, were contained in the syrup-residue, it would be taken up by the alcohol and thus separated from the glycerin-phosphoric acid compound, the latter body being insoluble in alcohol. The alcohol-hydrochloric acid extract, after treatment with animal charcoal, was reduced to a great concentration and a solution of platinum chloride added, which gave a heavy, voluminous, yellow precipitate, soluble in water. The water solution of the platinum double-salt of the base was treated with hydrogen sulphide, the platinum sulphide removed, and the solution evaporated to a small volume. The aqueous solution of the hydrochloride of the base gave reactions with the given reagents which are strictly characteristic of cholin:

With phosphotungstic acid,	white precipitate.
" phosphomolybdic "	yellow "
" potassium-bismuth-iodide, red	" "
" iodine-potassium-iodide, brown	" "
" chloride of gold,	yellow " soluble in water.

The residue, from which the cholin had been separated, was examined in respect of the presence of phosphoric acid, and the latter was identified by the use of ammonium molybdate. It has thus been established that the organic phosphorus-compound which was separated from the No. 3 stage germinated material by extraction with ether and alcohol-ether was a lecithine, which has been observed by the identification of its decomposition products.

Before reviewing the analytical data which have been given in respect of the conclusions which are indicated, certain observations may be included bearing upon the dissimilar behavior of the glycerides and the lecithines under simple mechanical pressure. It has been found that when the cotton seed is submitted to pressure for obtaining the oil, the normal glycerides become separated, and that the same quantity of lecithines which is found in the mature seed is found in the material from which the glycerides have been expressed. The observation was made with the cotton seed meal, in the form of cattle-food cakes, and the following data were obtained :

Material.	Extract. Per cent.	Lecithine in material. Per cent.	Lecithine in extract. Per cent.
Mature seed,	40.764	0.94	2.306
Cotton seed meal,	13.45	1.06	7.92

The normal and the substituted glycerides have shown dissimilar behavior under the respective actions of artificial solvents, mechanical pressure and germination.

In recapitulating the data obtained in the study of the given stages of germination or development of the seeds under investigation, certain conclusions are clearly indicated. It is apparent that the lecithine bodies are formed in the young plantlet during the initial and later stages of development. To exemplify this observation more clearly, the relative amounts of lecithines in the mature seed, and in the last stage of germination, give conclusive testimony. In the example of *Phaseolus vulgaris*, 100 parts of the water-free mature seed are equal to about 75 parts of the water-free No. 2 stage germinated material. It has been seen that the

relations between the lecithine amounts in the mature and germinated seeds are as follows:

Mature seed = 0.933 per cent.

No. 2 germinated = 3.230

But as 75 parts of germinated material are the product of 100 parts of mature seed, the 3.23 per cent. found in the former material is reduced to 2.42 per cent., which shows that during the initial stages of growth, under normal conditions of light, heat and moisture, the lecithine bodies had become increased from 0.933 per cent. to 2.42 per cent., or by 159.0 per cent. In the examples of the cotton seed and maize the same observations have obtained, which are specially emphasised in the instance of *Zea mays*. The analytical data attaching to the cotton seed indicate that the maximum of the lecithine contents of the organism was reached in the No. 1 stage of the germination, and that in the later stages the plantlet had reconsumed a portion of the lecithine store.

It is thus apparent that the plantlet, during the incipient period of growth, under the influence of light, is capable of utilising the inorganic phosphorus present in the mother-seed for the formation of new lecithine bodies; and further, that the plantlet, at a later stage, and when it is becoming independent of the mother-seed, decomposes and utilises those bodies in its continued development. It has been said that the plantlet is capable of utilising the inorganic phosphorus in the organisation of the lecithines. That is made very clear by the observation that the period of organisation is during the first stage of growth, and during the time that the plantlet is deriving its whole support from the mother-seed.

The observations which have been made are not conclusive, however, that the whole of the lecithine bodies found in the young plantlet are the products of reorganisation. That portion in the young plantlet which is in excess of the proportion contained in the mature seed is most clearly a reorganisation product. It is possible, on the other hand, and the observations made in the study of the earlier stages of the germination make it appear probable, that all that portion of the lecithine bodies contained in the mother-seed passes over into the organism of the young plantlet without decomposition or change and becomes directly available for its development. The latter question, as to whether the lecithines

resist the action of the ferments of the vegetable organisation during germination and pass over from the mother-seed into the plantlet undecomposed, will be the subject of further research, and in connection with the further question whether the lecithines present in animal organisms are organised by the animal organs or taken up direct from vegetable substances.

XVIII.—PINE TREE HONEY-DEW AND PINE TREE HONEY.

WITH NOTES ON THE NATURE OF THE DEXTROGYRATE BODIES IN HONEY.

BY H. W. WILEY.

In March, 1890, I received from Mr. W. M. Evans, of Amherst, Va., a sample of pine tree honey-dew and of pine tree honey.

The honey-dew, according to the description sent by Mr. Evans, was collected drop by drop from the pine leaves. At the same time Mr. Evans sent me a sample of pine tree honey which he says was, without doubt, made by the bees from the pine leaves during the winter, since no other honey-producing plants were accessible to them. Having seen it stated in some of the foreign journals that honey made from pine forests gave a right-handed polarisation, I thought it would be of interest to examine the two samples sent by Mr. Evans to determine, if possible, their nature. The small quantity of the honey-dew which I had at my disposal made, of course, a thorough study impossible. So far as could be determined, however, it had the following characteristics:

It contained 54.41 per cent. of water and 45.59 per cent. of solid matters. Calculated as dextrose it contained 17.44 per cent. of reducing sugar. After inversion it contained 26.03 per cent. of reducing sugar, which would indicate a content of sucrose equivalent to 8.16 per cent. At 31° by direct polarisation it gave an angular deviation equivalent to $36^{\circ}.56'$ of the cane sugar scale (Ventzke). If we assume that the reducing sugar present was pure invert sugar, then the levogyrate influence of 17.44 per cent. of invert sugar at 31° would be equal to $-4^{\circ}.97'$ of the cane sugar scale. Adding this number to the direct polarisation, it is seen that this would have amounted to 41.53 divisions of the scale.

Deducting from this number the right-handed polarisation due to the sucrose present, viz., 8.16, we have 33.37 divisions of right-handed rotation due to bodies other than sucrose present in the dew. The sum of the rotations of the invert sugar, viz., 17.44 per cent., and the sucrose 8.16 per cent., is 25.60 per cent. of the total solid matter due to sucrose and invert sugar. Deducting this number from the total solids present, viz., 45.59, there is left 19.99 per cent. solid matter which has caused the right-handed deviation of 33.37 divisions. This substance, therefore, has a specific rotatory power fully one-half greater than sucrose. It is not, therefore, due to pinite, but to some body or mixture of bodies having a specific rotatory power of $(a)_D = 105$ (circa.)¹ Having thus found that the pine tree exudation itself is a right-handed body, I naturally expected to find the honey made therefrom also right-handed; in this expectation, however, I was disappointed. The direct polarisation of the honey, under normal conditions at 31°, was — 13 divisions of the cane sugar scale, and the total reducing sugar which it contained, calculated as dextrose, was 70.42. Not only, therefore, was this sample of pine tree honey left-handed, but it was also left-handed to a degree greater than that ordinarily exhibited by honeys. From this fact the inference is to be drawn either that in gathering the honey the bees exerted upon it an inverting effect which made it left-handed, or else Mr. Evans was mistaken in supposing that the bees had derived their stock of honey from the trees in question.

The peculiar climatic conditions of last winter presented, through the whole season, the mildness of spring, affording, perhaps, an exceptional opportunity for the development of the pine tree honey-dew, and I am sorry that my attention was not called to the matter in time to have secured much larger quantities of this interesting substance.

The probable existence in honey of a right-handed body not sucrose or dextrose was indicated by me in a paper published in the "*American Apiculturist*," Vol. 3, No. 12, 1885. In this paper it is shown that a varying amount of substance, which I supposed then to be pure dextrin, was found in honey, amounting in this case to as much as 4 per cent.

Amthor has found² that honey gathered from pine forests con-

¹ This rotation would indicate that the body might be arabinose [$(a)_D = 105$], but there was not a sufficient amount of it definitely to determine this point.

² Rep. anal. Chem. 1885, 163.

tains dextrin, as he supposed at that time, even in such quantities as to become dextro-rotatory.

Klinger claims that this phenomenon is not confined exclusively to honey of coniferous origin. At that time I was led to express, in the paper referred to, my doubt of the truth of Lenz's observation¹ that after fermentation honey yields no optically-active substance. This doubt of mine has been confirmed by subsequent investigations.

Raumer² calls attention to the fact that the statement of Sieben, that the addition of starch syrup to honey could with certainty be detected by the residue of right-handed dextrin, is held to be unreliable. According to Sieben, 25 grams of honey dissolved in 150 cc. of water and treated with 12 grams pressed yeast (starch-free) are completely fermented in two days. The residue, clarified with aluminium hydrate and filtered, is completely inactive optically and does not reduce Fehling's solution. Even when treated with hydrochloric acid, to convert any starch or dextrin into dextrose, no reduction takes place. Raumer asserts that Sieben's observations must have been made on abnormal honeys.

Amthor has also called attention to the fact that honeys treated according to the method of Sieben, described above, showed right-handed polarisation, and that this phenomenon was supposed to be due to the honey having been largely gathered from pine forests. Honeys of known purity were found to be uniformly right-handed after fermentation. In three samples the dextrogyratory power, after fermentation, was expressed by the following numbers respectively: $2^{\circ}.83$, $1^{\circ}.58$, $2^{\circ}.70$. In three more samples of doubtful origin the numbers obtained were: $2^{\circ}.13$, $2^{\circ}.53$, $3^{\circ}.23$ respectively.

In the first three samples all possibility of adulteration is positively excluded. It was also established that the bees had not been fed with glucose. It is well known that feeding glucose or ordinary sugar causes foul brood. Only pure rock candy can be used for such purposes.

It was next deemed of interest to determine the nature of this dextrogyratory body. In order to purify the fermentation residue as completely as possible the method of Schmidt for obtaining the so-called gallisin was employed.³

¹ Chem. Ztg. 8, 613.

² Ztschr. angew. Chem.; 1889, 607.

³ Ber. d. chem. Ges. 13, 1000.

The honey in 10 per cent. solution, after fermentation, was filtered and evaporated to a thin syrup and poured into absolute alcohol. The brownish precipitate thus obtained was rubbed with absolute alcohol, the liquid poured off, and the residue rubbed twice with 96 per cent. alcohol, separated on a filter, and treated three times with ether. The residue was next dissolved in a small quantity of water, treated with bone-black and filtered. If the water-clear solution is now poured into absolute alcohol, a snow-white precipitate is formed. When the precipitate has subsided the alcohol is removed with a siphon and the residue treated again with a mixture of water-free ether and absolute alcohol. After 36 hours it was found that the precipitate had settled well to the bottom. The liquid was poured off and the residue poured into a large asbestos filter previously weighed. The washing was performed three times with absolute alcohol and three times with ether, and a stream of dried hydrogen conducted over the residue, which caused it to fall into a pulverulent mass. Finally, it was dried in hydrogen at 60°. At this temperature the mass fell together into a lump. The filter was further dried 48 hours over sulphuric acid and weighed in a closed tube. The specific rotatory power for four samples of the substance, prepared as above, from four different kinds of honey, was as follows:

From Nürnberg honey, ...	68.06
" " "	59.30
From honey from head gardener, Seyfferth, ...	58.30
From Nürnberg honey, ...	52.60

The reducing power of the body for Fehling's solution was determined for the first and second samples, both before and after inversion.

No. 1.

Before inversion, 1 gram substance reduced .455 gram copper.
After inversion, 1 gram substance reduced 1.200 gram copper.

No. 2.

Before inversion, 1 gram substance reduced .3048 gram copper.
After inversion, 1 gram substance reduced .845 gram copper.

It is evident from the foregoing that no single substance composes the unfermentable residue of honey, and it is further evident that this substance is not largely composed of dextrin. It was further established that the substance, after inversion, was fully

fermentable. Judged by its specific rotatory power above, this substance resembles Schmidt's gallisin, but its reducing power is quite different. The researches show that the purity of a honey cannot be safely determined by the fermentation process as practiced heretofore.

In regard to this right-handed body in honey, Amthor and Stern¹ have made some interesting investigations.

44.9655 grams of a sample of undoubtedly genuine honey were dissolved in 300 cc. water and allowed to ferment with yeast. After filtering and reducing to 200 cc. the liquid was examined in a Laurent polariscope and gave $+24^{\circ}.9$ in a 20 cm. tube. The dextrin was then converted into dextrose by heating 100 cc. with 10 cc. hydrochloric acid for three hours in a water-bath. After cooling and making up again to 100 cc., the polarisation gave $9^{\circ}.21$, equal to 2.249 per cent. dextrose. Allihn's gravimetric process gave 2.266 per cent. The formation of dextrose points to the honey having contained dextrin. The authors further confirmed this view by preparing phenylglucoson. A large quantity of honey was fermented in a weak solution; the filtrate yielded, on evaporation, a brown, thickish syrup, which was really an impure dextrin. After dissolving in a little water, phosphotungstic acid, containing some sulphuric acid, was added and the liquid filtered. The excess of reagent was removed by means of baryta water, and this in turn by the cautious addition of sulphuric acid. After concentrating, a little *liquor plumbi* was added, the liquid filtered, and the excess of lead removed by means of hydrogen sulphide. The filtrate was evaporated in the water-bath until it became a thin syrup, and 200 cc. of 90 per cent. alcohol were added, which was sufficiently diluted to keep the dextrin in solution. After filtering and evaporating, the dextrin was obtained as a brown syrup, which was purified with animal charcoal, until it was finally obtained as a yellowish, brittle, amorphous mass.

To obtain the related glucose, 5 grams of this dextrin were dissolved in 200 cc. of water mixed with 20 cc. of 20 per cent. sulphuric acid, and heated for three hours on the water-bath. The liquid was neutralised with barium carbonate, filtered and evaporated to a syrup, which was repeatedly exhausted with alcohol. After distilling off, a brownish syrup was obtained, which was purified by animal charcoal.

¹ Zeitschr. angew. Chem. 1889, p. 575.

It crystallises in granules, reduces Fehling's solution strongly, and the authors succeeded in forming phenylglucoson by warming 1 gram with 2 grams of phenylhydrazin, 3 grams of sodium acetate and 20 cc. of water. The crude product was recrystallised from alcohol and obtained in small yellow needles fusing at 204° C. This removes all doubt about the original substance being dextrin, the presence of which in honey may perhaps be explained by the supposition of the bees feeding, when they get a chance, on malt in breweries.

From a careful study of the above data it is shown that the honey-like exudation of the pine tree, without doubt, differs in a marked degree from that of the honeys of ordinary plants in being right-handed, from a polariscopic point of view, and containing bodies not sucrose nor invert sugar, with a specific rotatory power of about 105. It appears further that, according to the observations of some authors, honey made from the exudation of pines is naturally right-handed, though this observation is not confirmed by the single sample which I had at my disposal.

I should be glad hereafter to receive samples of honey of undoubted pine tree origin, for the purpose of making a further study in regard to its nature, and I hope to take advantage of the next mild winter to obtain more extensive samples from the localities affording those samples already examined.

Up to the present time it must be confessed that no genuine sample of honey collected in this country has shown a right-handed rotation. Honey possessing this property therefore greatly excites suspicion, and warrants an inquiry concerning its composition.

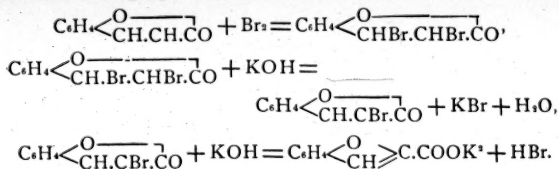
ON COUMARONE.

By A. R. L. DOHME.

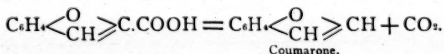
This substance was first made by Fittig and Ebert,¹ who obtained it by the distillation of coumarilic acid with lime, in the course of their work upon lactones and in particular upon the lactone coumarin. By treating this latter substance with bromine

¹ Ann. Chem. (Liebig) **216**, 168.

and heating with alcoholic potash the dibrom addition-product thus formed, they obtained *a*-bromcoumarin, which, upon further treatment with caustic potash, yielded coumarilic acid, according to the following equations:



The transformation of coumarilic acid into coumarone, with loss of carbon dioxide, is represented by the following equation:



This represents Fittig's view of the constitution of coumarone, regarding it as a closed ring and as bearing the same relation to indol that furfuran bears to pyrrol. Rössing¹ also, somewhat later, obtained coumarone in the course of his work upon ortho-aldehydephenoxycetic acid, from which he, however, first obtained coumarilic acid. Fittig found it to be a slightly yellow, highly refracting oil, possessing a characteristic aromatic odor, insoluble in water but quite easily volatile with water-vapor, completely insoluble in alkalis, and hence, in all probability, no phenol. Nascent hydrogen and fuming hydrobromic acid or hydriodic acid do not act upon it, while concentrated sulphuric acid causes it to change to a reddish-brown amorphous mass. Bromine, however, was found by these investigators to act quite readily upon the oil, yielding a dibrom addition-product which, upon treatment with boiling alcohol or alcoholic potash, yielded an oil volatile with water-vapor, and crystallising, upon cooling, to a mass of prisms which melt at 36° and are monobromcoumarone. Owing to lack of material they here left the subject, and no work was done upon it until Kraemer and Spilker,² during the past year, found that coumarone was a constant constituent of the light oils obtained in the distillation of coal-tar. Having thus found a ready means of obtaining coumarone in any desired quantity, they proceeded to make some experiments with it.

¹ Ber. d. chem. Ges. 17, 3000.

² Ibid. 23, 78.

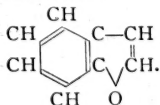
repeating and verifying Fittig's work and trying the action of chlorine upon it. By the action of the latter reagent they obtained an oil boiling at 245° – 248° , which is dichlorcoumarone, analogous to the dibrom addition-product obtained by Fittig. By treatment with alcoholic potash they also obtained the corresponding monochlorcoumarone, which melts at 74° – 75° and boils at 215° – 217° . The tarry product obtained by the action of concentrated sulphuric acid upon coumarone they name "paracoumarone," finding it to be a polymer of coumarone. Their synthesis of chrysene by heating together coumarone and naphthalene is also interesting, inasmuch as it shows that the oxygen atom of coumarone is removable without breaking down the entire molecule. Before the work of Kraemer and Spilker was published, the writer, at the suggestion of Prof. A. W. von Hofmann, undertook to experiment upon coumarone, hoping to get some well-crystallised derivatives, and from the reactions of these to throw some light upon the constitution of the substance. First, the action of aniline at all temperatures from 190° to 280° in sealed tubes was tried, but without any positive result, as most of the coumarone was changed to paracoumarone and the aniline remained unchanged. Concentrated ammonia was next tried, in the hope of replacing the oxygen atom by the imido-group and thus obtaining indol, but, even at 280° in sealed tubes, there was no sign of a reaction. The dibrom addition-product was made by treating the dry oil with bromine, when the former changes from an oil to a solid mass of white crystals. The melting point observed was 88° , which agrees with Fittig's and with Kraemer's figures. From this, monobromcoumarone was made by treating the dibromide with alcoholic potash. The mixture was distilled with steam, and the colorless oil that came over soon solidified to a mass of white crystals which melted at 36° , the melting point observed by Fittig. The action of aniline at 200° was tried upon this monobromcoumarone, in the hope of introducing the group C_6H_5NH in the place of the bromine atom. There was no reaction, however; even at 280° in sealed tubes no change could be observed in the contents of the tube, the monobromcoumarone separating out as such on adding water, while silver nitrate failed to show the presence of any trace of hydrobromic acid. Concentrated ammonia also did not act upon the monobromcoumarone when the two were heated together at 280° . The action of phosphorus pentachloride upon coumarone was

next tried, in the hope of thus replacing the oxygen atom, and from the resulting product drawing some conclusion as to the constitution of the substance. With this reaction in mind, molecular quantities of phosphorus pentachloride and coumarone were heated in sealed tubes at 175° , after it had been shown that no reaction took place when the constituents were heated even at 200° with an inverted condenser. The product of the reaction was a brown-colored oil, the reaction having generated a considerable quantity of hydrochloric acid, which, when the tube was opened, escaped under great pressure. The oil was fractionated and yielded three fractions, boiling respectively at 70° – 100° , 210° – 220° and 220° – 250° , there being, however, very little of the latter formed. After repeated fractionation, the first fraction was recognised as phosphorus trichloride, as it boiled at 74° – 76° , while the second proved to be monochlorcoumarone, which had been obtained by Kraemer and Spilker, and which boiled at 216° – 218° . The third fraction was, in all probability, some dichlorcoumarone, which, as found by the just mentioned authors, boils at 245° . In all probability, then, the phosphorus pentachloride has merely acted as a chlorinating agent and given up its two loosely-held chlorine atoms to the coumarone. Thus dichlorcoumarone was formed, which, at the high temperature prevailing, lost hydrochloric acid and formed monochlorcoumarone, the small portion of dichlorcoumarone found having probably not taken part in the latter reaction. Raising the temperature from 175° to 280° did not yield any products different from those obtained at 175° , so that it was clear that the oxygen atom of coumarone is certainly not loosely held in combination in the molecule. Next it was thought possible to remove this oxygen atom by heating the monochlorcoumarone just obtained with phosphorus pentachloride at a high temperature in a sealed tube, and thus obtain orthochlortrichlorstyrene, or, if addition also took place, orthochlorotetrachlorethylbenzene. Five grams of the monochlorcoumarone were sealed in a tube with an excess of phosphorus pentachloride and heated at 275° for five hours. The product of the reaction was a brown-colored oil, which, upon distillation, yielded two products, one boiling at 75° , and hence being phosphorus trichloride, and the other boiling at 255° – 260° . Hydrochloric acid was generated in quantity. The latter fraction, upon cooling, showed signs of crystallisation, and, being soluble in ether with great readiness, was set aside in a solution of the latter

reagent to crystallise. This it did in long, thin, white, satiny needles which melted at 57.5° – 58° . Not very much of this, however, was obtained, and after two futile attempts to make a chlorine estimation according to Carius, the tubes exploding each time, the very small amount of the substance left was analysed and gave approximate figures for a dichlorocoumarone of the formula $C_8H_4Cl_2O$, $(C_6H_4 < \overset{O}{\underset{||}{C}} Cl > CCl)$.

0.1808 gram substance gave 0.2810 gram AgCl, which is equivalent to 38.4 per cent. chlorine, whereas the formula $C_8H_4Cl_2O$ requires 37.96 per cent. chlorine. Owing to the close of the semester, no more of the substance could be made, and the verification of this substance will have to be postponed until a future investigation, when it is proposed also to study the action of phosphorus pentasulphide upon coumarone, inasmuch as a preliminary experiment showed that a reaction takes place between these substances. For when the oily product obtained by heating them together in a sealed tube at 180° , is distilled with steam, no coumarone could be detected in the receiver, thus showing that it must at least have been changed by the reaction, even if substitution had not taken place, for coumarone is very volatile with water-vapor.

The object in view in these experiments was to replace the oxygen atom of coumarone, and from the resulting compounds or reactions to deduce some argument for or against the Fittig-Hantzsch formula for the substance:



In so far as the experiments have any value whatever, being mostly negative, they tend to confirm rather than contradict this formula; for the stability of the coumarone molecule certainly was quite striking in the course of the work, and the difficulty experienced in attempting to replace the oxygenation also spoke in favor of the closed-ring formula for the substance. In conclusion, I would like to thank Prof. von Hofmann for his kindness to me and for the instruction I have received while working under his supervision.

I. CHEMICAL LABORATORY, UNIVERSITY OF BERLIN, March, 1890.

ON THE QUESTION OF CONCORDANCE IN ATOMIC WEIGHT DETERMINATIONS.

BY F. W. CLARKE.

In the determination of atomic weights the subject of constant errors has of late years received much attention. On the experimental side, that is with reference to individual series of data, the possibility of constant error is always carefully considered; but another phase of the question, involving antecedent values, is too apt to be ignored. Under the latter heading one special case is familiar to all chemists, namely, the uncertainty in the ratio between oxygen and hydrogen, in consequence of which we have two distinct standards for atomic weights, the one $H=1$, the other $O=16$. The difference between these standards is small in itself, but among the higher atomic weights it becomes multiplied to serious proportions. In other words, if the atomic weight of hydrogen be taken as unity, the uncertainty in the atomic weight of oxygen becomes a large constant error in the calculation of other values. This particular error is merely a type of its class; for whenever an atomic weight is computed from other atomic weights which are assumed to be known, their errors, together with the experimental errors of the determinations themselves, are accumulated in the final result. Thus it sometimes happens, when an atomic weight has been determined by different methods, involving the use of different compounds, that the concordance or discordance between the several series of data obtained may depend upon the factors assumed in the final calculation. These factors are themselves subject to errors, great or small, which become constant errors in calculation, and which, although cumulative in their effects, are commonly neglected altogether. Only those determinations of atomic weight which depend upon direct comparison of an element with the standard of value are free from errors of this class.

In a recent paper upon the atomic weight of cadmium, by E. A. Partridge,¹ the foregoing suggestions are well exemplified. Experimentally Mr. Partridge's work appears to be excellent, and on their face his results are concordant in the highest degree. Three methods were adopted for the determinations as follows: first,

¹Am. Jour. Sci., Nov. 1890, p. 377.

the ignition of CdC_2O_4 to CdO ; second, the conversion of CdSO_4 into CdS by heating in a stream of H_2S ; third, a similar conversion of CdC_2O_4 into CdS . In each series ten experiments were made, and from each experiment individually the atomic weight of cadmium was computed. In brief, the results obtained were as follows, omitting details and taking $\text{O} = 16$ as the standard of comparison:

	$\text{CdC}_2\text{O}_4 : \text{CdO}$.	$\text{CdSO}_4 : \text{CdS}$.	$\text{CdC}_2\text{O}_4 : \text{CdS}$.
$\text{Cd} = \text{maximum,}$	111.834	111.818	111.833
minimum,	111.759	111.778	111.771
mean,	111.8027	111.7969	111.805

The mean of all is $\text{Cd} = 111.8015$, with an extreme range from lowest to highest of only 0.075.

Curiously, however, Mr. Partridge does not state what values he assumed for the atomic weights of carbon and sulphur in making his computations. Working back from some of his figures, I find that he must have taken the round numbers 12 and 32; and just here the question of concordance may be raised. In a recalculation of the data I have reduced all the experiments to percentage form, getting the averages of the three series into the subjoined proportions:

1st series, $\text{CdC}_2\text{O}_4 : \text{CdO} :: 100 : 63.964 ; \pm .0011$.

2d series, $\text{CdSO}_4 : \text{CdS} :: 100 : 69.199 ; \pm .0012$.

3d series, $\text{CdC}_2\text{O}_4 : \text{CdS} :: 100 : 71.973 ; \pm .0007$.

Now, when $\text{O} = 16$, these ratios give equations containing each an absolute term. If we compute with $\text{C} = 12$ and $\text{S} = 32$, we get one set of values for Cd ; if we take Stas' figures, $\text{C} = 12.005$ and $\text{S} = 32.074$, we get another set; and if we assume the factors given in my own "Recalculation of the Atomic Weights," viz., $\text{C} = 12.001$ and $\text{S} = 32.058$, we have still a third series. The results are as follows, in mean:

	$\text{C}, 12. \quad \text{S}, 32.$	$\text{C}, 12.001. \quad \text{S}, 32.058.$	$\text{C}, 12.005. \quad \text{S}, 32.074.$
1st series, $\text{Cd} =$	111.801	111.8036	111.8178
2d series, $\text{Cd} =$	111.785	111.7271	111.7111
3d series, $\text{Cd} =$	111.808	111.6054	111.5788
Range,	.023	.1982	.2390

Hence we see clearly that the concordance between the three series of determinations is conditioned, under the ordinary modes of calculation, by the uncertainties in the factors used. If the

concordance shown by Partridge is real, Stas' values for C, or S, or both, are in error. If Stas' values are correct, then the three series of Mr. Partridge are discordant by nearly one-fourth of a unit in the atomic weight of cadmium. The small variations in the low atomic weights are multiplied in the higher figure.

In a discussion of this sort it is perhaps not possible to establish any distinct criterion of accuracy by means of which constant errors can be detected and placed. It is possible, however, at least in certain cases, to so handle data that the results of calculation shall involve no extraneous uncertainties. In the present instance the ratios measured by Partridge are capable of quite independent discussion, and by the simplest of means.

If we continue to put $O = 16$ as our standard of comparison, and use the symbols of the elements algebraically to represent atomic weights, the three proportions given above may be transformed into the following linear equations:

$$\text{1st series, } 36.036\text{Cd} = 127.928\text{C} + 2493.696.$$

$$\text{2d series, } 30.801\text{Cd} = -30.801\text{S} + 4428.736.$$

$$\text{3d series, } 28.027\text{Cd} = 143.946\text{C} - 100\text{S} + 4606.272.$$

Here we have three equations with three unknown quantities, Cd, C, and S, and no assumption need be made in advance as to the value of either. Solving directly, these equations give us the following results:

$$\text{Cd} = 111.7850$$

$$\text{C} = 11.9958$$

$$\text{S} = 32.0002.$$

These values are independent of all other determinations, and contain only the errors of Partridge's own work, divided into three portions, instead of being accumulated upon the atomic weight of cadmium alone. They involve, however, the assumption that the three sets of observations are of equal weight, which may or may not be true. If different weights are assigned we must then form normal equations by the method of least squares, and their solution might modify our final figures. For present purposes, however, the more laborious method of computation, with its discussion of weights and probable errors, is quite unnecessary. Such a discussion may be found in Strecker's recalculation of Liebig and Redtenbacher's data relative to the atomic weight of carbon.¹ So far as I know, that paper stands alone in chemical literature, the only one of its kind.

¹ Ann. Chem. (Liebig) 59, 280 (1846).

Recurring now to the three atomic weights deduced from the three equations, we find that the values for carbon and sulphur vary slightly from the best recognised determinations. The extent of that variation may perhaps serve as an index of the accuracy of the work done, and the question of concordance of data assumes a new form. The more nearly the figures for C and S agree with established values, the better the determinations probably are; and in the present case the agreement is close enough to establish a reasonable presumption in favor of Partridge's methods. The constant errors in his work cannot be large, and they are probably all in one direction.

But between Partridge's determinations, however discussed, and the atomic weight of cadmium as measured by either Dumas or Huntington, there is a discordance of nearly half a unit. The latter chemists, both working with haloid salts of cadmium, are in close agreement; and their data, with Stas' values for Ag, Cl, and Br, and with $O=16$, make Cd about 112.23, very nearly. This discordance is probably real, and due mainly to differences in experimental methods. Its cause can only be found by further experiments, and certain lines of investigation are easily suggested. Apart from the obvious plan of scrutinising each method by itself, and seeking for the constant errors which must exist on one side or the other, it is clear that new modes of determination should be attempted, lying between and connecting the existing data. For example, the ratios $CdO : CdSO_4$, $Cd : O$, $Cd : S$, $Cd : Ag$, $Cd : Cl$, and $Cd : Br$ are all apparently capable of direct measurement; and they would, if determined, serve to check each other and to control the older determinations. A system of such interlacing ratios can be discussed collectively, by least squares, so as to distribute errors of observation, the constant error of any one series of data being equivalent to an accidental error in its relations to the other series. In a large system of ratios the constant errors can hardly be all in one direction, and they must tend to neutralise each other to a very considerable degree. Each ratio should contribute to our knowledge of every atomic weight involved in it, and not be considered solely with reference to a single component. The current methods of computation are fallacious in so far as they tend to magnify and accumulate errors which could be partially eliminated.

ON BENZOQUINONECARBOXYLIC ACIDS.

BY JULIUS STIEGLITZ.

Only one compound of the group of benzoquinonecarboxylic acids is known, viz., trimethylquinonecarboxylic acid, $(\text{CH}_3)_3\text{C}_6\text{O}_2(\text{COOH})$, obtained by J. U. Nef¹ by oxidising *p*-diamidodurylic acid, $(\text{CH}_3)_2\text{C}_6(\text{NH}_2)_2(\text{COOH})$. All attempts to obtain such acids from their well-known and stable ethers by saponification, as well as from hydroquinonecarboxylic acids by oxidation, were unsuccessful. As yet, however, only such ethers of quinone-carbonic acids have been investigated as contain ethylated carboxylic groups directly attached to the benzene ring, and which therefore are β -ketonic acid ethers. As different results might be obtained with quinone compounds of the γ - and δ -ketonic acid series, I began an investigation of the benzoquinone derivatives of malonic ether. A method for the preparation of these derivatives of malonic ether was suggested by the ease with which halogen atoms in certain quinone compounds had been replaced by various radicals.

Paradichlorquinonedimalonic Ether, $\text{C}_6\text{Cl}_2\text{O}_2 \left(\text{CH} < \begin{smallmatrix} \text{CO}_2\text{C}_2\text{H}_5 \\ \text{CO}_2\text{C}_2\text{H}_5 \end{smallmatrix} \right)_2$,

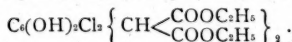
was prepared by treating chloranil (1 molecule) in dry benzene or acetone solution with sodium malonic ether (4 molecules) in absolute-alcoholic solution. The reaction, which takes place instantly in the cold, is completed by warming a few minutes on a water-bath. By acidifying, washing, drying and evaporating the benzene solution, a dark oil results, which deposits yellow needles when mixed with a little cold alcohol. After recrystallisation from hot alcohol, light yellow needles are obtained, which have the composition $\text{C}_{26}\text{H}_{22}\text{Cl}_2\text{O}_{16}$.

	Calculated.		Found.	
	I.	II.	I.	II.
C_{26}	240	48.68	48.43	...
H_{22}	22	4.46	4.55	...
Cl_2	71	14.40	...	14.35
O_{16}	160	32.46	...	...
	493	100.00		

The proof that the two chlorine atoms replaced occupied *para* positions is given below.

¹Ann. Chem. (Liebig) 237, 11.

A great many experiments were made to determine the best conditions for the reaction, but only a small yield could be obtained—in no case more than 10 per cent. of the theoretical yield. No other pure compounds could be separated from the dark mother-liquid. Dichloroquinonedimalonic ether is difficultly soluble in cold alcohol, ether or benzene, and dissolves most readily in chloroform and hot alcohol. It dissolves in sodic and potassic hydrate solution with a deep pure violet color resembling exactly a concentrated solution of potassium permanganate. The coloration is due to the formation of salts in which the remaining acid hydrogen atom of the malonic ether groups is replaced by sodium or potassium. These ether salts are rapidly saponified in the cold, as will be described below, without any change of color. With ammonia the new compound gives a deep navy-blue solution whose color disappears after some time. Reducing agents transform it into paradichlorhydroquinonedimalonic ether,



The reduction can be effected by sulphurous acid in aqueous or acetone solution, or by zinc dust and glacial acetic acid. By the latter method a green product results, from which white crystals can be obtained by recrystallising from alcohol and washing with cold ether. The white needles formed melt at 160°–161° to a perfectly colorless liquid;¹ they are easily soluble in alcohol, ether, benzene, etc., and in ammonia with a transient deep blue color, in fixed alkalies with a permanent dark violet color. Their composition is $\text{C}_{20}\text{H}_{24}\text{Cl}_2\text{O}_{10}$.

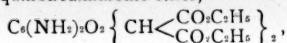
	Calculated.		Found.
	I.	II.	
C ₂₀	240	48.48	48.30
H ₂₄	24	4.85	4.87
Cl ₂	71	14.34	...
O ₁₀	160	32.33	...
	495	100.00	

Oxidising agents, as ferric chloride, convert the hydroquinone into the quinone. Dichloroquinonedimalonic ether was further characterised by the replacement of its two chlorine atoms by amido-groups, by treating it with alcoholic ammonia in the

¹ In marked contrast to the analogous dichlorhydroquinoneterephthalic ether. Hantzsch, Ber. d. chem. Ges. 20, 1313.

cold. When the dark-blue coloration first formed is converted into a dark-yellow one, the reaction is finished.

Paradiamidoquinonedimalonic ether,



is precipitated when the yellow alcoholic solution is diluted with water and poured into very cold dilute sulphuric acid. The red needles obtained can be purified by dissolving them in chloroform and precipitating them with petroleic ether or simply by washing with cold ether. They melt at 159° – 160° and appear as long, flat, perfect prisms when examined with a microscope. They are soluble in alkaline, but not in acid, solvents. A combustion showed that the formula $\text{C}_{20}\text{N}_2\text{H}_{26}\text{O}_{10}$ is correct.

	Calculated.	Found.
N	6.17	6.07

Saponification of dichlorquinonedimalonic ether.—When this ether is suspended in an excess of dilute sodic hydrate it dissolves very quickly, and soon the smell of alcohol is unmistakably perceptible; if, after standing only *a few minutes*, the solution is poured into cold sulphuric acid, a brown precipitate is formed. By recrystallisation from acetone or alcohol dark-red plates are obtained, which sublime with slight decomposition and without melting, and which are easily soluble in alkalis, alcohol and acetone, less so in ether. The compound is parachlorhydroxyquinone, $\text{C}_6(\text{OH})\text{H}_2\text{ClO}_2$.

	Calculated.		Found.	
	I.	II.	I.	II.
C ₆	72	45.43	45.17	...
H ₂	3	1.89	2.10	...
Cl	35.5	22.39	...	22.19
O ₂	48	30.29	...	...
	158.5	100.00		

The relative position of the chlorine atom and the hydroxyl-group is shown by the following experiment. If, as just described, dichlorquinonedimalonic ether is dissolved in an excess of dilute alkali, the solution allowed to stand for *an hour* and then warmed for a few minutes on a water-bath, both chlorine atoms are replaced by hydroxyl-groups. The brown alkaline liquid is slowly poured into strong sulphuric acid, which is kept cold by ice so that its

temperature never rises above 15° ; if this solution is extracted with a large quantity of ether, the ether leaves upon evaporation crystals of paradihydroxyquinone.

The crystals were compared with a specimen prepared from dihydroxyquinone terephthalic ether,¹ and were found to be perfectly identical with Nietzki's and Löwy's paradihydroxyquinone. The compound obtained from dichlorquinonedimalonic ether sublimed between 190° and 205° ; it dissolved readily in acetic ether, from which it was obtained in the saw-edged crystals characteristic of the substance. With concentrated alkalis a red precipitate was formed, which gave with a little water a red, with more water a yellow, solution. The quinone itself had an orange color and was found to be absolutely free from chlorine. The yield was too small to make a combustion possible, but all qualitative tests agreed with the properties of paradihydroxyquinone, the formation of which is also indicated by the results obtained by analysing the chlorhydroxyquinone obtained by the same reaction. The formation of paradihydroxyquinone proves that all the compounds described are para-derivatives of benzene.

The reaction by which chlorhydroxyquinone and dihydroxyquinone are produced must consist in the replacement of the malonic groups by hydrogen; by a reaction analogous to that in the case of the replacement of the formyl rests by hydrogen in Löwy's reaction, after the chlorine atoms in dichlorquinonedimalonic had been replaced by hydroxyl groups. This unexpected result has led me to begin an investigation of the product formed by the action of sodium malonic ether on dibromquinoneterephthalic ether. As yet no definite results have been obtained in this line of investigation, and I shall only add a few words about the preparation of dibromquinoneterephthalic ether, as I could greatly simplify Herrmann's² and Böniger's³ method for its preparation from succinylosuccinic ether. The latter is dissolved in dry chloroform, and bromine, in the proportion of equal molecules, gradually added; the chloroform solution is then shaken with an aqueous solution of sulphurous acid, dried and evaporated on a water-bath. The crude dihydroxyterephthalic ether obtained is dissolved in warm glacial acetic acid, and water (5-10 per cent. of the weight of glacial acetic acid used) and 3 more

¹ Löwy: Ber. d. chem. Ges. **19**, 2385; Böniger: Ibid. **22**, 1284; Nietzki: Ibid. **21**, 2374.

² Herrmann: Ber. d. chem. Ges. **19**, 2234.

³ Böniger: Ibid. **21**, 1758.

equivalents of bromine added. This mixture is allowed to stand over night in a closed flask. Dibromquinoneterephthalic ether separates out in crystals, and more of it is precipitated by successive additions of small quantities of water. It is generally perfectly pure, but if it should contain some unchanged dihydroxyterephthalic ether, it can be easily purified by dissolving in a small quantity of hot glacial acetic acid, cooling, and adding some water and a little bromine. A second method consists in dissolving the succinylosuccinic ether directly in glacial acetic acid and treating with bromine and water in one operation; it is advisable then to add the water only after about a third of the bromine has been already used. 30 grams of succinylosuccinic ether gave, by the first method, 40.5 grams of pure dibromquinoneterephthalic ether (84.5 per cent. of the calculated quantity); and 10 grams gave, by the second method, 13.2 grams of the dibrom-compound (82.5 per cent. of the theoretical yield). Professor Nef proposed to me to try new methods for making benzoquinonecarboxylic acids, and helped me by his advice, which I wish here to acknowledge with thanks.

CHEMICAL LABORATORY, CLARK UNIVERSITY.

Contributions from the Chemical Laboratory of Wesleyan University.

IX.—THE ACQUISITION OF ATMOSPHERIC NITROGEN BY PLANTS.

BY W. O. ATWATER AND C. D. WOODS.

REPORTED BY W. O. ATWATER.

[Continued from page 547, Vol. 12.]

Loss of Nitrogen.

In many of the experiments, instead of gain of nitrogen, a decided loss was observed. With peas, the loss appears in every case in which there were no root tubercles, and in many of the cases in which only a "few" tubercles were developed. (See Table 4 above, and detailed tables at end of this article.) This loss of nitrogen manifested itself whether the plants were fed with nitrogen

or not, though it was larger with the nitrogen-fed plants than those which received no nitrates.

With the oats and corn (see Tables 1 and 2 above, and detailed tables at the end of article), most of the nitrogen-fed plants showed a large loss, while with those which had no nitrates there was a slight gain.

Two sources of loss of nitrogen suggest themselves, namely, decomposition of the organic matter of seed or plant, and decomposition of nitrates fed. Loss from decomposition of seed, or of seed and plants, accords with the results of observations in experiments at this place, from the report¹ of which the following table is copied. In each of the thirteen experiments, from five to twenty-three peas were germinated in a germinating apparatus or in sand, and allowed to grow for a longer or shorter period in water or sand without artificial food supply. The difference between the nitrogen in the seed and that found at the end of the experiment in the plants and the medium in which they grew is the nitrogen lost.

Experiments on the Loss of Nitrogen during Germination and Early Growth.

Conditions of Germination and Growth of Peas.	Experiments.		Time of germination and growth.	Nitrogen lost in per cent. of total in seed at beginning of experiments.
	Series.	No.		
<i>Water Culture.</i>				
Seeds germinated and then suspended with roots immersed in pure water. Experiments in open air, . . . }	III. {	3	8 days.	8.4 per cent.
		1	10	9.8
		2	15	1.5
<i>Sand Culture.</i>				
Seeds germinated and grown in sand. Experiments in open air, . . . }	IV. {	6	6	5.9
		7	10	10.6
		8	14	15.1
Seeds germinated and then transferred to sand, { Experiments in greenhouse, }	V. {	9	18	7.3
		10	28	12.4
	VI. {	11	52	16.3
		12	29	8.2
{ Experiments in dwelling-house, }		13	42	11.0

¹ On the Loss of Nitrogen by Plants during Germination and Growth: W. O. Atwater and E. W. Rockwood, this Journal 8, 327.

The results thus cited indicate that germination and early growth of peas are, or at any rate may be, accompanied by greater or less loss of nitrogen. In the experiments herewith reported upon there was an average loss of about 9 milligrams of nitrogen with the peas (Series VII and IX) which were not nitrogen-fed and which failed to develop root tubercles. In the peas of Series VII and IX which were fed with nitrates there was an average loss of about 23 milligrams in the plants without root tubercles. The loss with the nitrogen-fed peas was two and one-half times as great as with those not nitrogen-fed.

With oats and corn, fed with very little or with no nitrogen, there is no apparent loss, but a small gain instead. On the other hand, with the nitrogen-fed oats and corn the loss of nitrogen is marked, increases with the quantity of nitrogen supplied, and is much larger than with the peas.

This loss of nitrogen gives added force to the suggestion made in the report¹ of the earlier work here, that if nitrogen escaped in some of the trials it may have done so in the other cases as well. If this be true the results are all inaccurate as indications of the actual atmospheric nitrogen acquired, and the plants must really have obtained more nitrogen than the figures here imply.

These experiments do not tell to what extent the loss observed with the oats and corn and with the peas which had no root tubercles, was from the seed and to what extent from the nitrates; whether, as would seem most likely, it was due to the action of microbes; or what connection there may be between plants of different species and the loss of nitrogen. These and kindred questions must remain for future research to decide.

Acquisition of Nitrogen from the Air.

That the legumes experimented with obtained large amounts of nitrogen from the air is abundantly shown by the facts cited in the tables. The evidence coincides exactly with that of the previous experiments here and the later ones by Hellriegel and others, and is plain and unmistakable. Peas of a small, early variety (East Hartford Early), grown in sand, with no nitrogenous food except that in the seed and what they were able to acquire from the atmosphere, attained a height of over five feet. With nitrogen supplied in the nutritive solutions they reached a height of over

¹ Loc. cit. p. 403.

eight feet. The quantities of atmospheric nitrogen obtained by the pea and alfalfa plants (Tables 3, 4 and 5 above) were in many cases very large.

Whether the nitrogen thus obtained from the air was free or combined nitrogen these experiments do not absolutely prove. But the quantities obtained are so *very* large as to leave little doubt that it is free nitrogen. That any considerable quantity of combined nitrogen could have been taken from the atmosphere by the nutritive solutions and soil, and thus conveyed to the plants, would seem from the consideration cited in connection with the descriptions of "blank" tests above, to be out of the question, nor does it seem at all reasonable to assume the possibility of so much combined nitrogen being absorbed by the foliage of the plants.¹ The experiments of Hellriegel already referred to, in which plants grown in confined air without combined nitrogen, but with root tubercles, acquire considerable quantities of nitrogen, would seem to put the acquisition of free nitrogen by plants beyond question. This special subject, however, demands further investigation. Plans for its study are now being made.

One special point calls for notice here. In general, the smaller plants with fewer seeds show a loss of nitrogen, while the larger, stronger plants with more normal development show the gain. In many of the experiments which demonstrated the gains of nitrogen, some of the plants were large and had a number of well-developed seeds, while others were small and had few seeds. The natural inferences are that the gain of nitrogen was made by the well-developed plants; that the actual gain was larger than the apparent gain; and that this actual gain by the well-developed plants covered the apparent gain in the whole experiment *plus* enough to make up for any losses from decomposition of seeds of both the vigorous and the weaker plants, from decomposition of nitrates, and from other causes. In other words, it seems probable that the vigorous plants had to make up for the losses of the weaker ones, and hence the actual gain by normal plants is larger than the statistics of these experiments show.

Thus in the experiments with East Hartford Early Peas it was observed that, as a rule, unless the plants not supplied with nitrates made a growth of at least 14 inches and developed more

¹ See discussion of this subject in description of previous experiments in this place, this Journal 6, 378.

than one pea to a vine, the pea was a small one, and the results indicated a loss of nitrogen. The following data from Table 9 will serve as illustrations:

No. 120—1 plant, 15 inches high; 1 pod, 1 pea; loss, 7.7 mg. nitrogen.

No. 128—3 plants, 12 inches high; 3 pods, 3 peas; loss, 3.6 mg. nitrogen.

No. 137—3 plants, 10-15 inches high; 1 pod, 1 pea; loss, 11.4 mg. nitrogen.

No. 138—4 plants, 12-27 inches high; 2 pods, 2 peas; loss, 3.9 mg. nitrogen.

No. 140—4 plants, 9.12 inches high; 4 pods, 4 peas; loss, 2.4 mg. nitrogen.

In only one case (No. 127; 3 plants, 12-15 in. high; 3 pods, 3 peas; gain, 26.5 mg. nitrogen) did the plants not nitrogen-fed show a gain without producing more than an average of one pea per vine. In this case the 15-inch plant had two peas, and it seems at least reasonable to question whether this plant did not make the gain.

In nearly all, if not all, of the experiments with East Hartford Early Peas that have shown a gain of nitrogen, some of the plants have been small and with no peas, or at most only one. It seems reasonable to suppose from the above-cited trials that the apparent gain may have been due to the large, strong plants, and that these latter gained enough to offset the loss by the weaker plants and provide, in addition, for the apparent gain of the whole experiment. The following from table above cited will illustrate.

No. 136 had four plants; three 14 inches and one 58 inches high and bearing 5 pods with 12 peas. The estimated gain of nitrogen was 242.9 milligrams. In this case the three 14-inch plants bore 3 pods with 3 peas, and the large plant 2 pods with 9 peas. The loss almost uniformly observed with the small plants bearing few peas in other experiments, makes it possible that the small plants did not hold their own; that, in other words, taken by themselves they lost nitrogen. If this be so, the gain of at least one-fourth of a gram must have been made by the one large plant. The same may be said of No. 135. Two of the plants were small and bore practically no fruit. The apparent gain was about one-fifth gram. The inference is that the well-developed plants made the whole gain of nitrogen. In most of the experiments there was

more or less of this difference of growth, and to distribute the gain evenly to all of the plants of a single experiment would obviously be erroneous. Indeed, there is little room for doubt that the apparent gain of the large plants would have been materially greater than that of the whole experiment, if the weak plants had not been present.

Why, in the same pot, under apparently the same conditions, one plant should thrive so much better than others; why one should grow luxuriantly while others starve, is difficult to understand. That this was the case in the experiments with East Hartford Early Peas is evident. The growth of Champion of England Peas has been much more uniform. The different plants in the same pot differ but little from each other in apparent vigor of growth.

The plan for the next experiments here will include pots with only one plant in each, and thus eliminate the difficulties noticed in the experiments with East Hartford Early Peas, and to a greater or less extent in those with other plants.

Species of Plants which acquire Atmospheric Nitrogen in Large Quantities.

In our experiments, peas and alfalfa have gained large quantities of nitrogen from the air. The corn in Series IX acquired small quantities of nitrogen from the air, but this acquisition was not substantiated with experiments of corn and oats of Series XII and XIII. The reasons for this difference of results with the corn and oats demand further experiment for their explanation.

Hellriegel,¹ Wolff,² Bréal,³ Lawes and Gilbert,⁴ and others have found peas, lupines, serradella, beans, and, in all probability, clover and other legumes to acquire atmospheric nitrogen, while the cereals, buckwheat and potatoes, have failed to give positive evidence of so doing. The facts thus attested accord with manifold observations of field practice in implying that leguminous plants, or at least some species of legumes, possess in high degree the power to obtain nitrogen from the air.

Relation between Root Tubercles and Gain of Nitrogen.

Examination of the detailed tabular statements of results of 80 experiments with peas and alfalfa here described shows that in

¹ Loc. cit.

² Compt. rend. 100, 670.

³ Landw. Jahrb. 1887, 653.

⁴ Loc. cit.

every case, without exception, where there were no root tubercles there was loss of nitrogen; where there were "few" tubercles there was sometimes a slight loss of nitrogen, at other times a slight gain; with a "fair number" of tubercles there was a decided gain; where there was a "large number" of tubercles the gain of nitrogen was very large.

In the experiments with corn and oats, which had no tubercles, there was, in a few cases, a very slight gain of nitrogen, in others a loss, generally very large. The explanation of this behavior of the cereal plants must await further investigation. Meanwhile it is sufficient to say the leguminous plants have root tubercles and acquire large quantities of nitrogen.

The nature and functions of root tubercles, though now actively discussed, are not fully understood. While there is as yet no absolute proof that the root tubercles or the microbes which appear to be connected with them are the cause of the gain of nitrogen, the fact that there is a connection between the root tubercles and the amount of nitrogen acquired by the plants from the air is unmistakable.

It seems probable that this relation holds in field as in pot culture. The past season the Station grew half an acre of cow-peas, which yielded at the rate of about eight tons of green fodder per acre. In some ten different places in the field the roots were examined and found to be covered with tubercles of considerable size. Some were as large as peas. At one end of the field, where the yield was relatively light, the roots had less tubercles than elsewhere; and in general where the growth was heaviest, the tubercles seemed to be most abundant.

Our experiments thus confirm the important observation, first made by Hellriegel, of the connection between root tubercles and acquisition of nitrogen, and support the belief that the faculty of obtaining atmospheric nitrogen is especially characteristic of some, if not all, species of legumes.

Relation between Soil Infusions and Root Tubercles.

As may be seen from Table 6, herewith, the addition of soil infusion seemed to make infection much more certain, but a large number of the plants had an abundance of root tubercles without the infusions, although grown in sterilised sand supplied with water that had been distilled, and solutions prepared with that

water. That this should be the case is not at all surprising if, as seems probable, the development of the tubercles is due to bacteria or other micro-organisms. These experiments were conducted near cultivated ground, a garden, where such organisms or their germs might naturally be abundant in the air and find their way to the pots in which the plants were cultivated.

A possible explanation of this greater abundance in the nitrogen-fed plants may be that these plants are sustained by the nitrogen supplied until they become infected and are enabled to attain the nitrogen from the air.

TABLE 6.

Relation between Soil Infusion and Tubercles.

NUMBERS OF EXPERIMENTS IN WHICH THERE WERE NO ROOT TUBERCLES, A FEW, A FAIR NUMBER, OR A LARGE NUMBER, AS ESTIMATED AT HARVESTING.

	Champion of En. land Peas, Series VII, 1888.	Alfalfa, Series VIII.	East Hartford Early Peas, Series X, 1889.	Champion of England Peas, Series XI, 1889.	Total No. of Experiments.	Per Cent. of Total No. of Experiments.
<i>Without Soil Infusion.</i>						
Experiments with no tubercles, ...	2	...	1	2	5	15.6
" " few tubercles, ...	2	2	2	2	8	25.0
" " fair number of tubercles,	1	...	2	1	4	12.5
" " large number of tubercles,	1	...	11	3	15	46.9
Total number of experiments, ...	6	2	16	8	32	100.0
<i>With Soil Infusion.</i>						
Experiments with no tubercles, ...	0	...	1	0	1	2.7
" " few tubercles, ...	2	1	9	1	13	35.1
" " fair number of tubercles,	2	1	2	2	7	18.9
" " large number of tubercles,	4	1	6	5	16	43.3
Total number of experiments, ...	8	3	18	8	37	100.0

Table 6 recapitulates the results of 69 experiments, 64 with peas (those with bacteria cultures are omitted) and 5 with alfalfa. The first group contains 32 experiments with peas and alfalfa, to which no soil infusions were added. The last column shows the per-

centage of experiments in which tubercles were found at harvest. The plants in 47 per cent. of the experiments had a "large number" of tubercles, notwithstanding they received no soil infusion. The second group contains 37 experiments with alfalfa and peas, all of which were treated with soil infusion. In only 43 per cent of these were a "large number" of tubercles found.

SUMMARY.

The results of the experiments above described, and the conclusions drawn from them and from the other investigations referred to, may be briefly summarised as follows:

1. In the previous experiments at this place (made in 1881-2 and published in 1882, 1884 and later), peas were shown to obtain large quantities of nitrogen from the air during the period of growth. The conditions were such as to make it extremely probable that the free nitrogen of the air was thus assimilated.

2. Later experiments by Hellriegel and others have confirmed the results thus obtained with peas; have shown similar acquisition of nitrogen from the air by serradella, lupine, clover and other legumes; have strongly confirmed the assimilation of free nitrogen, and have shown a connection between root tubercles and the acquisition of atmospheric nitrogen.

3. The experiments here described still further confirm the acquisition of atmospheric nitrogen by peas, and show that the same is done by alfalfa.

4. In the eighty-nine experiments here reported, there was in no case any large gain without root tubercles, but with them there was uniformly more or less gain of nitrogen from the air. As a rule, the greater the abundance of root tubercles, the larger and more vigorous were the plants and the greater was the amount of atmospheric nitrogen acquired. The connection between root tubercles and the acquisition of nitrogen, which was first pointed out by Hellriegel, is abundantly confirmed. What this connection is; what are the relations of micro-organisms to the root tubercles and to the acquisition of nitrogen; and in general how the nitrogen is obtained, are questions still to be solved.

5. In the experiments here reported the addition of soil infusions did not seem necessary for the production of root tubercles. A plausible supposition is that the micro-organisms or their spores were floating in the air and were deposited in the pots in which the plants grew.

6. The cereals here experimented with did not manifest this power of acquiring nitrogen, nor did they have such tubercles as are formed on the roots of legumes. These results accord with those obtained elsewhere, except in the fact that in some of the trials very small quantities of nitrogen were apparently acquired from the air. What importance, if any, is to be attached to this observation it is not easy to say.

7. In a number of these experiments, as in similar ones previously reported, there was a loss of nitrogen instead of gain. The loss occurred where there were no root tubercles; it was especially large with oat and corn plants, and largest where they had the most nitrogen at their disposal in the form of nitrates. This loss may probably be due to the decomposition of the seeds and nitrates through the agency of micro-organisms.

8. The failure of earlier experiments to bring evidence of the acquisition of nitrogen from the air may very probably be due in part to this loss of nitrogen, but more especially to the precautions for excluding compounds of nitrogen, which precautions were likewise calculated to exclude the action of microbes, electricity, or both, by the aid, or at least in the presence, of which the nitrogen is assimilated.

In brief, the acquisition of large quantities of atmospheric nitrogen by leguminous plants, which was first demonstrated by experiments here and has been since confirmed by others, is still further confirmed by the experiments herewith reported. These experiments in like manner confirm the observation of the connection between root tubercles and the acquisition of nitrogen. There is scarcely room for doubt that the free nitrogen of the air is thus acquired by plants.

MIDDLETOWN, CONN., *April*, 1890.

APPENDIX.

The detailed results of the experiments described above are condensed in Tables I-VII which follow.

TABLE I.—*Series VII, 1888. Champion of England Peas.*

CONDENSED STATISTICS.

No. of Experiment.	Kilos. of Sand.	Weight of Seed.	Nitrogen Supplied.			Soil Infusions.	Water added during Growth.	Root Tubercles at Harvest.	Mature Plants.				Nitrogen at End of Experiment.			Gain (or Loss —) of Nitrogen.		
			In Seed.		Total.				Number.	Weight.		In Plants.	In Nutrients.	Total.				
			Mg.	Mg.						Tops.	Roots (and Sand).				Total.		Mg.	Mg.
81	2.8	1111.5	48.9	...	48.9	Cc.	700	None.	3	2.26	1.06	G.	3.32	41.9	...	41.9	Mg.	77.0
82	2.8	1048.5	46.1	...	46.1	...	600	Few.	3	1.03	2.07	G.	3.10	44.4	...	44.4	...	1.7
83	2.8	1059.0	46.6	...	46.6	25	900	Fair number.	3	2.78	2.55	G.	5.33	132.2	...	132.2	...	85.6
84	2.8	1020.5	44.9	...	44.9	25	900	Large number.	3	2.64	1.79	G.	4.43	101.6	...	101.6	...	116.7
86	2.8	1047.5	46.1	75.9	122.0	...	1000	Fair number.	3	2.55	5.75	G.	8.30	138.1	24.8	162.9	...	40.9
87	2.8	979.9	43.1	75.9	119.0	25	1100	Few.	3	2.59	4.18	G.	6.77	136.3	25.6	161.9	...	42.9
88	2.8	1054.0	46.4	75.9	122.3	25	800	Fair number.	3	2.30	1.81	G.	4.11	124.6	24.2	148.8	...	26.5
89	2.8	1090.5	47.9	151.8	199.7	...	600	Few.	3	2.21	2.37	G.	4.58	122.3	57.2	179.5	...	20.2
91	2.8	1064.0	46.8	151.8	198.6	25	1100	Large number.	3	3.30	2.00	G.	5.30	216.5	64.0	280.5	...	81.9
92	2.8	1030.0	45.3	151.8	197.1	25	1000	Large number.	3	2.93	1.31	G.	4.24	183.6	69.4	253.0	...	55.9
93	6	1070.0	47.1	113.8	160.9	...	1300	None.	3	3.07	5.16	G.	8.23	135.0	17.0	152.0	...	8.9
94	6	1031.5	45.4	113.8	159.2	...	1700	Fair number.	3	2.56	1.20	G.	3.76	122.8	28.2	151.0	...	8.2
95	6	1063.5	46.8	113.8	160.6	25	1700	Large number.	3	2.31	1.27	G.	3.58	131.6	38.1	169.7	...	9.1
96	6	969.5	42.7	113.8	156.5	25	900	Few.	3	.68	.84	G.	1.52	44.7	95.0	139.7	...	16.8

TABLE II.—Series X, 1889. *East Hartford Early Peas.*

CONDENSED STATISTICS.

No. of Experiment.	Kilos. of Sand.	Weight of Seed.	Nitrogen Supplied			Water added during Growth.	Duration of Growth.	Root Tubercles at Harvest.	Mature Plants.					Nitrogen at End of Experiment.		Gain (or Loss) — of Nitrogen.	
			In Nutritive Solutions.						Height.	Number of Pods.	Number of Peas.	Weight.			In Nutritive Solutions.		Total.
			In Seed.	In Nutritive Solutions.	Total.							Vines and Fruit.	Roots (and Sand).	Total.			
		Mg.	Mg.	Mg.	Cc.	Days			Inches.	G.	G.	G.	Mg.	Mg.	Mg.		
115	1	511	20.4	...	...	1525	75	Large number.	2 15,	1.37	.67	2.04	33.0	33.0	33.0	12.6	
116	1	486	19.4	...	...	1525	75	Few.	1 20,	2	.92	1.14	25.4	25.4	25.4	6.0	
117	1	487	19.5	...	...	1575	80	Large number.	2 16, 32	1.66	.43	2.09	48.7	48.7	48.7	29.2	
118	1	483	19.3	...	...	1375	75	Few.	2 12, 16	2	.77	.25	1.02	23.8	23.8	4.5	
119	1	526	21.0	...	...	1675	75	Large number.	2 18, 18	3	1.57	.51	2.08	52.5	52.5	31.5	
120	1	480	19.2	...	...	1400	75	None.	1 15,	1	.41	.25	.66	11.5	11.5	7.7	
121	1	491	19.6	75.9	75.9	2150	75	Few.	2 24, 30	3	3.90	.55	4.45	100.2	100.2	4.7	
122	1	486	19.4	75.9	75.9	2000	70	Few.	2 24, 27	3	3.73	.68	4.41	90.9	90.9	4.4	
123	1 1/2	706	28.2	...	...	2250	75	Large number.	3 2, 12, 15	2	1.00	.65	1.65	30.7	30.7	2.5	
124	1 1/2	720	29.0	...	...	2350	75	Large number.	3 6, 15, 15	4	2.19	.74	2.93	69.5	69.5	40.4	
125	1 1/2	744	29.8	...	...	2350	75	Large number.	3 12, 12, 15	3	1.82	.65	2.47	56.3	56.3	26.5	
126	1 1/2	707	30.7	...	...	2150	70	Few.	3 12, 12, 12	3	1.45	.55	2.00	7.1	7.1	3.6	

129	1 1/2	770	30.8	129.0	159.8	...	4900	84	Large number.	322, 27, 48	412	7.85	1.07	8.92	186.2	7.8	194.0	34.2	
130	1 1/2	758	30.3	129.0	159.3	...	5450	97	Fair number.	323, 54, 81	3	7	7.17	8.11	153.2	18.7	171.9	12.6	
131	1 1/2	745	29.8	129.0	158.8	...	5900	99	Large number.	235, (27, 86) ¹	512	10.05	1.98	12.03	202.2	1.9	204.1	45.3	
132	1 1/2	788	31.5	75.9	107.4	40	3250	69	Few.	314, 21, 27	413	5.28	1.20	6.48	99.1	4.7	103.9	-3.6	
133	1 1/2	776	31.0	129.0	160.0	40	5600	97	Large number.	319, 22 ¹ , 30, 84	823	11.03	1.60	12.63	277.1	1.7	278.8	118.8	
134	1 1/2	710	28.4	75.9	104.3	40	3200	69	Few.	318, 24, 27	513	4.90	1.58	6.54	102.9	...	102.9	-1.4	
135	3	998	39.9	...	39.9	...	6600	90	Large number.	414, 14, 30, 62	925	7.97	1.27	9.24	237.1	...	237.1	197.2	
136	3 1/2	940	37.6	...	37.6	...	7600	127	Large number.	414, 14, 14, 58	512	7.06	3.74	10.80	280.5	...	280.5	242.9	
137	2 1/2	958	38.3	...	38.3	...	3250	75	None.	310, 15, 15	1	.97	1.55	2.52	26.9	...	26.9	-11.4	
138	3	1013	40.5	...	40.5	...	5050	75	Few.	412, 12, 15, 27	2	2	1.42	1.12	2.54	36.6	...	36.6	-3.9
139	3	970	38.8	...	38.8	50	5050	113	Fair number.	413, 15, 15, 44	4	6	2.81	.65	3.46	67.5	...	67.5	28.7
140	2 1/2	981	39.2	...	39.2	50	3250	70	Fair number.	4	9, 12, 12, 12	4	1.57	1.20	2.77	36.8	...	36.8	-2.4
141	3	1006	40.2	113.8	154.1	...	7600	113	Fair number.	418, 24, 52, 54	616	8.53	3.31	11.84	154.6	2.1	156.8	2.7	
142	3	1001	40.0	113.8	153.9	...	6000	83	Large number.	418, 22, 26, 72	925	11.62	2.17	13.79	273.2	...	273.2	119.3	
143	3	1023	40.9	113.8	154.7	...	4700	75	Large number.	424, 27, 27	613	5.98	1.57	7.55	153.0	3.7	156.7	1.9	
144	3	973	38.9	113.8	152.7	50	5000	75	Few.	322, 22, 22, 22	413	6.05	2.20	8.25	140.9	2.5	143.4	-9.3	
145	2 1/2	975	39.0	113.8	152.8	50	5800	83	Large number.	418, 30, 78	928	9.08	1.33	11.31	196.5	...	196.5	43.7	
146	3	952	38.1	113.8	151.9	50	5700	83	Fair number.	310, 15, 30, 30	718	6.85	1.82	8.67	211.1	4.6	215.7	63.8	
147	3 1/2	1005	40.2	227.6	267.8	...	5600	70	Few.	421, 27, 27, 27	818	9.10	2.80	11.90	221.8	26.4	248.2	-19.6	
148	3 1/2	1046	41.8	227.6	269.4	...	10400	127	Large number.	430, 30, 41, (102, 102) ¹	411	14.88	3.27	18.15	409.3	5.3	414.6	145.2	
149	3 1/2	1017	40.7	227.6	268.3	50	9100	99	Large number.	424, 33, 84 (18, 96) ¹	1126	15.00	2.80	17.80	260.3	1.6	261.8	-6.5	
150	3 1/2	954	38.2	227.6	265.8	50	8200	97	Large number.	416, 35, 42, 83	1124	12.40	3.60	16.00	319.1	32.9	332.0	87.2	

¹ One of the plants had two branches of the heights given.

EXPLANATION OF TABLE.—This series includes three groups of experiments, Nos. 115-124, with 1/4 or 1 kilogram of sand; Nos. 125-134, with 1/2, and Nos. 135-150, with 2 1/2, 3 or 3 1/2 kilograms of sand. The plants of Nos. 115-150 were grown without addition of nitrogen other than that which was contained in the peat-soil infusion, and were watered with peat-soil infusion and three times with water. The plants of Nos. 125-134, were watered with a small proportion of nitrogen as nitrates, and the plants of Nos. 135-150, with a relatively large proportion of nitrogen as nitrates. Half received peat-soil infusion to include four experiments, Nos. 128, 129, 130, and 131. Nos. 128 and 129, received a small proportion of nitrogen as nitrates, and the plants of Nos. 130 and 131, received a relatively large proportion of nitrogen as nitrates. Half received peat-soil infusion to include four experiments, Nos. 132, 133, 134, and 135. Nos. 132 and 133, received a small proportion of nitrogen as nitrates, and the plants of Nos. 134 and 135, received a relatively large proportion of nitrogen as nitrates. In the third group, five, Nos. 141-146, had a small proportion of nitrates, and five, Nos. 136-140, had none. Nine of this group were treated with peat-soil infusion.

TABLE III.—Series XI, 1889. *Champion of England Peas.*

CONDENSED STATISTICS.

Experiment.			Nitrogen Supplied.			Mature Plants.					Nitrogen at End of Experiment.								
Kilos. of sand.	Weight of Seed.	In Seed.	In Nutritive Solutions.		Total.	Soil Infusions.	Water added during Growth.	Days	Tuberles at Harvest.	Weight.				In Plants.	In Nutritive Solutions.	Total.			
			Mg.	Mg.						Number.	Extremes of Height.	Number of Pods.	Number of Peas.				Vines and Fruit.	Roots (and Sand).	Total.
151	3.1581	72.3	...	...	72.3	Cc.	4200	78	None.	4	25-34	G.	3.37	1.68	5.05	Mg.	62.4	...	62.4
152	3.1554	70.9	...	...	70.9	Cc.	4300	79	None.	2	30-39	G.	3.03	2.25	5.28	Mg.	62.8	...	62.8
153	3.1564	71.5	...	...	71.5	Cc.	4100	79	Few.	4	33-40	G.	2.69	1.26	3.95	Mg.	58.0	...	58.0
154	3.1553	71.0	...	...	71.0	Cc.	4200	85	Fair number.	4	36-46	G.	5.72	2.45	8.17	Mg.	145.8	...	145.8
155	3.1553	73.3	...	...	73.3	Cc.	4700	85	Large number.	4	41-50	G.	5.99	2.74	8.73	Mg.	151.9	...	151.9
156	3.1606	75.2	...	...	75.2	Cc.	4800	85	Fair number.	4	32-48	G.	5.42	1.72	7.14	Mg.	139.1	...	139.1
157	3.1543	70.7	151.8	...	222.5	Cc.	7500	83	Fair number.	4	44-51	G.	12.47	7.96	20.43	Mg.	282.7	...	282.7
158	3.1559	70.2	151.8	...	222.0	Cc.	7600	85	Large number.	4	54-62	G.	14.52	3.84	18.36	Mg.	343.8	...	343.8
159	3.1541	70.7	151.8	...	222.6	Cc.	8000	85	Large number.	4	58-64	G.	14.38	4.67	19.05	Mg.	340.0	...	340.0
160	3.1532	70.9	151.8	...	222.7	Cc.	8000	85	Large number.	4	43-60	G.	12.49	4.00	18.95	Mg.	411.4	...	411.4
161	3.1588	72.6	151.8	...	224.3	Cc.	7900	85	Few.	4	42-54	G.	10.95	2.08	13.03	Mg.	236.8	...	236.8
162	3.1643	75.0	151.8	...	226.8	Cc.	8000	85	Large number.	4	42-62	G.	12.62	3.52	16.14	Mg.	284.9	...	284.9
163	3.1405	109.9	447.7	...	557.6	Cc.	12500	85	Few.	6	48-68	G.	25.52	9.56	35.08	Mg.	593.6	...	593.6
164	6.2241	102.4	447.7	...	550.1	Cc.	13000	85	Large number.	6	48-68	G.	27.67	10.75	38.42	Mg.	672.7	...	672.7
165	6.2300	105.1	447.7	...	552.8	Cc.	13900	85	Large number.	6	44-70	G.	24.85	7.87	32.72	Mg.	694.5	...	694.5
166	6.2286	104.5	371.8	...	476.3	Cc.	8900	54	Large number.	6	42-64	G.	17.27	6.67	23.94	Mg.	105.5	...	105.5

1 g. peas eaten by birds, 2 g. peas eaten by birds, 3 g. peas eaten by birds, 4 Growth stopped when plant was in full bloom.

EXPLANATION OF TABLE.—In this series the plants in one group of six pots (Nos. 151-156) were grown without addition of nitrogen other than that in the seed. In six other pots (Nos. 157-162) a larger pot was added in the form of a nutrient. Half of the plants were treated with 29.6 g. of peat oil infusion in each pot in each of these six groups. The column "Exhaustion of Height" is the height of the shortest and of the tallest plant in each pot is given. The column "Weight of Root" and "Total weight of plant" are the weight of the root and of the whole plant respectively to remove. When about half-grown, some of the plants in Nos. 156, 157, and 158 were cut down, which was not practicable to remove. Gain in the last column is less than the actual gain. The column "Corrected gain" is the gain in this last column less the gain in the column "Weight of Root" and "Total weight of plant" respectively to remove. The attempt to drive them out with a small quantity of borax was successful, but the plants were killed thereby.

TABLE IV.—*Series VIII, 1888-89. Alfalfa.*

CONDENSED STATISTICS.

No. of Experiment.	Kilos. of sand.	Weight of Seed. ¹	Nitrogen Supplied.				Water added during Growth.	Root Tubercles at Harvest.	Mature Plants.				Nitrogen at End of Experiment.			Gain of Nitrogen.		
			In Seed. ¹	In Nutritive Solutions.					Number.	Branches.	Height.	Weight.		In Plant.	In Nutritive Solutions.		Total.	
				1888.	1889.	Total.						Tops.	Koots (and Sand).					Total.
109	12	..	Mg. ..	Mg. ..	Mg. ..	Cc. 50	Cc. 8700	Fair number.	3	16	Inches. 12-20	G. 3.22	G. 7.76	G. 10.98	Mg. 137.5	Mg. 137.5	Mg. 137.5	
111	12	..	..	113	227.6	340.6	..	8000	Few.	1	14	18	5.13	5.83	10.96	223.0	121.9	344.9
112	12	..	..	113	227.6	340.6	..	11100	Few.	1	13	28	10.27	10.12	20.39	440.0	109.8	549.8
113	12	..	..	113	227.6	340.6	50	12700	Large number.	1	35	20	12.90	18.45	31.35	618.4	74.3	722.8
114	12	..	..	113	227.6	340.6	50	9400	Few.	3	20	10-20	5.63	7.97	13.60	251.0	128.0	379.0

¹ The weights of the seeds and nitrogen supplied in them were very small and are omitted.

EXPLANATION OF TABLE.—In July, 1888, a number of experiments with alfalfa were started. The plants developed very slowly, and at the approach of cold weather were housed for the winter, during which season they made practically no growth. In the spring of 1889, the sand in each pot in which the plants had survived was extracted with water, the nitrogen (in nitrate-) determined, and the difference between the amount of nitrogen thus found and that originally supplied in the nutritive solutions charged to the plants. The plants were transplanted into pots containing freshly purified sand and allowed to grow during the spring and summer. In pots Nos. 111-113 two plants each died. The nitrogen in the plants which died was not estimated, hence whatever nitrogen they may have taken from the nitrates supplied is counted as equivalent to so much lost. Only five experiments were carried to a successful completion, one without and four with the addition of nitrogen as nitrates. The plants of three of these experiments, Nos. 109, 113 and 114, were each treated with 25 cc. of an infusion of soil in which alfalfa had been growing luxuriantly and 25 cc. of pea-soil infusion.

TABLE V.—Series XII, 1889. Oats.

CONDENSED STATISTICS.

No. of Experiment.	Nitrogen Supplied.			Soil Infusions.	Water added during Growth.	Duration of Growth. ¹	Root Tubercles at Harvest.	Mature Plants.							Nitrogen at End of Experiment.			Gain (or Loss) of Nitrogen.
	In Seed.	In Nutritive Solutions.	Total.					Number.	Height.	No. of Vats.	Weight.			In Plants.	In Nutritive Solutions.	Total.		
											Vines and Fruit.	Roots (and Sand).	Total.					
Kilos. of Sand.	Mg.	Mg.	Mg.	Cc.	Days				Ft.	G.	G.	G.	Mg.	Mg.	Mg.			
167	6 4.1	...	307.7	4100	84	None on any of the Plants.		6	1	7	.56	1.31	7.2	...	7.2	3.1		
168	6 4.2	...	307.8	4100	84			6	1	7	.68	.58	1.26	5.8	...	5.8	1.6	
169	6 4.3	...	307.8	4100	84			6	1	8	.72	.62	1.34	6.2	...	6.2	1.9	
170	6 3.8	...	307.8	4100	84			6	1	6	.69	.56	1.25	6.5	...	6.5	2.7	
171	6 4.2	303.5	307.7	7300	84	6	3	...	9.94	9.70	19.64	177.5	19.7	197.2	-111.5			
172	6 4.3	303.5	307.8	8000	84	6	3	...	11.29	4.96	16.25	205.5	8.1	213.6	-94.2			
173	6 4.3	303.5	307.8	8300	84	6	3	...	11.60	7.60	19.20	197.1	11.8	208.9	-98.9			
174	6 4.3	303.5	307.8	8300	84	6	3	...	10.50	8.50	19.00	160.9 ²	9.1	170.0 ²	...			
175	6 4.3	607.0	611.4	...	8600	84	6	3	...	15.80	6.10	21.90	391.7	25.7	417.4	-194.0		
177	6 4.3	607.0	611.4	50 9500	84	6	3	...	14.08	10.00	24.08	396.8	40.2	437.0	-174.4			
178	6 4.1	607.0	611.1	50 10600	84	6	3	...	13.62	10.19	23.81	332.2	23.8	356.0	-255.1			

¹ The plants in Nos. 167-170 had finished their growth, but the others were a little past the "milk" when harvested.² In the determination of nitrogen in plants a portion (estimated at one-fifth) was lost. The results were sufficient to indicate that there had been considerable loss of nitrogen in the experiment and to imply that the actual quantity of nitrogen in the plants was about the same as that of the others (Nos. 171-173) of this group.

EXPLANATION OF TABLE.—In Experiments Nos. 167-70, no nitrogen was added other than that contained in the seeds. Nos. 171-174 received a small proportion and Nos. 175-178 a larger proportion of nitrogen as nitrates. In each of six cases 50 cc. of pea-soil infusion were added.

TABLE VI.—Series IX, 1888. *Corn*.

CONDENSED STATISTICS.

No. of Experiment.	Kilos. of Sand.	Weight of Seed.	Nitrogen Supplied.			Soil Infusions.	Water added during Growth.	Root Tubercles at Harvest.	Mature Plants.				Nitrogen at End of Experiment.			Gain (or Loss) of Nitrogen.
			In Seed.	In Nutritive Solutions.	Total.				Number.	Weight.			In Plant.	In Nutritive Solutions.	Total.	
										Tops.	Roots (and Sand).	Total.				
48	9	929.5	Mg. 17.9	Mg. 17.9	Mg. 17.9	Cc. ...	Cc. 5900	None on any of the Plants.	2	G. 2.132	G. 5.287	G. 7.419	Mg. 21.6	Mg. ...	Mg. 21.6	Mg. 3.7
49	9	941.7	Mg. 18.1	Mg. ...	Mg. 18.1	Cc. ...	Cc. 5900		1	G. .176	G. 1.478	G. 1.654	Mg. 6.7	Mg. ...	Mg. 6.7	Mg. -11.4
25	16	942.0	Mg. 18.1	Mg. ...	Mg. 18.1	Cc. 25	Cc. 11300		2	G. 5.093	G. 8.028	G. 13.121	Mg. 38.1	Mg. ...	Mg. 38.1	Mg. 20.0
36	16	969.9	Mg. 18.6	Mg. ...	Mg. 18.6	Cc. 25	Cc. 10600		2	G. 4.272	G. 11.050	G. 15.322	Mg. 38.7	Mg. ...	Mg. 38.7	Mg. 20.1
26	16	979.5	Mg. 18.8	Mg. 113.8	Mg. 132.6	Cc. ...	Cc. 12700		2	G. 21.490	G. 26.800	G. 48.290	Mg. 170.5	Mg. ...	Mg. 170.5	Mg. 37.9
27	16	966.2	Mg. 18.6	Mg. 113.8	Mg. 132.4	Cc. ...	Cc. 12200	2	G. 23.960	G. 27.890	G. 51.850	Mg. 154.5	Mg. ...	Mg. 154.5	Mg. 22.1	
34	15	975.9	Mg. 18.7	Mg. 113.8	Mg. 132.5	Cc. 25	Cc. 11800	2	G. 21.730	G. 16.220	G. 37.950	Mg. 135.6	Mg. ...	Mg. 135.6	Mg. 3.1	
35	15	974.1	Mg. 18.7	Mg. 113.8	Mg. 132.5	Cc. 25	Cc. 12100	2	G. 21.260	G. 18.790	G. 40.050	Mg. 130.8	Mg. ...	Mg. 130.8	Mg. -1.7	

EXPLANATION OF TABLE.—In the first four experiments no nitrogen was added; in the last four experiments a small proportion was added in the form of nitrates. In one-half the cases 25 cc. of an infusion from the soil in which corn was growing were added in each case. In all of the experiments the quantity of food supplied to the plants was insufficient for the normal growth.

TABLE VII.—Series XIII, 1889. *Corn.*

CONDENSED STATISTICS.

No. of Experiment.	Kilos. of Sand.	Weight of Seed.	Nitrogen Supplied.			Water added during Growth.	Duration of Growth.	Root Tubercles at Harvest.	Mature Plants.					Nitrogen at End of Experiment.		Gain (or Loss—) Nitrogen.		
			In Seed.	In Nutrients.	Total.				Number.	Height.		Weight.		In Plants.	In Nutrients.		Total.	
										Inches.	Greatest.	Tops.	Koots (and Sand).					Total.
179	9	1150	21.1	...	21.1	Cc. 4800	166		2	10, 8	20, 23	3.60	5.55	9.15	31.2	Mg. 31.2	10.1	
180	12	1114	20.4	...	20.4	5000	107		2	5, 9	18, 28	4.85	4.60	9.45	35.8	15.4	6.0	
181	12	1183	21.6	...	21.6	5000	107		2	7, 8	16, 24	3.55	3.35	6.90	27.6	6.0	0.8	
182	12	1186	21.7	...	21.7	5000	107		2	7, 8	22, 22	3.60	3.80	7.40	31.5	9.8	0.8	
183	12	1114	20.4	...	20.4	5000	106		2	8, 8	24, 28	4.20	3.15	7.35	32.7	10.3	5.5	
184	12	1106	20.3	...	20.3	4900	106		2	10, 6	25, 22	2.85	2.60	5.45	25.8	5.5	5.5	
None on any of the Plants.																		
185	11	578	11.6	417.3	428.9	8700	127		1	45	56	22.85	13.42	36.27	323.9	105.0	...	
186	16	1081	19.8	607.0	626.8	...	127		2	57, 34	57, 43	48.85	12.27	61.12	509.1	4.1	...	
187	17	1098	20.1	607.0	627.1	13500	127		2	52, 57	52, 60	42.33	15.88	58.21	486.9	...	...	
188	17	1146	21.0	607.0	628.0	50 12600	127		2	52, 50	52, 60	51.72	17.51	69.23	535.3	...	...	
189	17	1126	20.7	607.0	627.7	50 13700	127		2	54, 49	54, 49	54.90	14.80	69.70	534.0	14.9	...	
190	17	1159	21.2	607.0	628.2	50 14100	127		2	46, 26	54, 43	50.07	18.93	69.00	569.9	...	...	
191	22	1111	20.4	1631.4	1651.8	...	127		2	60, 71	64, 79	94.00	20.35	114.35	1213.7	106.8	...	
192	22	1174	21.5	1517.6	1539.1	...	127		2	58, 63	58, 63	78.42	21.35	99.77	1185.2	79.9	...	
193	29	1179	21.6	1517.6	1539.2	...	127		2	64, 66	64, 66	89.03	34.82	123.85	1340.7	4.8	...	
194	22	1138	20.8	1517.6	1538.4	50 18400	127		2	61, 49	61, 59	74.33	21.80	96.13	1308.8	10.4	...	
195	22	1081	18.5	1517.6	1536.1	50 18300	127		2	66, 50	66, 50	67.04	29.14	96.18	1367.2	...	...	
196	30	1114	20.4	1517.6	1538.0	50 21900	127		2	46, 58	46, 58	75.30	37.85	113.15	1312.3	...	...	

EXPLANATION OF TABLE.—In Nos. 179-184 no nitrates were supplied; in Nos. 185-196 a large proportion of nitrogen was added in the form of nitrates. To one-half of the experiments of each group 50 cc. of an infusion of a soil in which corn was growing were added. Under "Height," the length of the plants is given at the time when they were harvested. Under "Weight," the weight of the plants is given at the time when they were harvested. Under "Gain (or Loss—) Nitrogen," the residual solutions, from time to time, implied that none of the plants had received enough food for normal growth.

ANALYTICAL DETAILS.

Determinations of nitrogen were made in seeds, nutritive solutions, residual solutions, and plants, by the method described under "Methods of Analysis" above. The details of the determinations of nitrogen in the plants are omitted here for the reason there given. The details of determinations of nitrogen in seeds, nutritive solutions and residual solutions follow.

Nitrogen in Seed. Method of Kjeldahl.

No. of Series.	Kind of Seed.	Weight of portion taken for analysis.	Nitrogen Found.		
			Weight.	Percent-age.	Average percentage
VII.	Champion of England Peas,	Mg.	Mg.		
		1001.0	43.75	4.37	
		1001.3	44.42	4.42	
		1002.0	43.96	4.39 ¹	4.39
IX.	Corn, ...	1001.0	18.98	1.90	
		1000.5	18.91	1.89	
		1002.0	19.27	1.92	
		1003.5	19.60	1.95	1.92
X.	East Hartford Early Peas,	1000.	39.90	3.99	
		1000.	40.19	4.02	
		1000.	40.19	4.02	4.01
XI.	Champion of England Peas,	1000.	45.62	4.56	
		1000.	45.80	4.58	
		1000.	45.80	4.58	4.57
XII.	Oats, ...	1000.	19.13	1.91	
		1000.	18.60	1.86	
		1000.	18.70	1.87	1.88
XIII.	Corn, ...	1000.	18.50	1.85	
		1000.	18.20	1.82	
		1000.	18.20	1.82	1.83

¹ Soda-lime method.

Nitrogen in "N. III" Solution, 1888.

25 cc. from "N. III" pipette { 37.69 mg. N.
37.64 " " } Average 37.66 mg. N.
37.64 " "

Nitrogen in "N. III" Solution, 1889.

25 cc. from "N. III" pipette { 37.93 mg. N.
37.87 " " } Average 37.94 mg. N.
38.03 " "

Nitrogen in Residual Solutions.

Series No.	Experiment No.	Liters of Extract.	Nitrogen found in one liter of Extract.			Total Nitrogen in residual solution.
			A.	B.	C.	
IX.	158	3	0.62	0.63		1.9
	159	3	0.68	0.65		2.0
	161	3	0.69	0.66		2.0
	163	4	0.57	0.63		2.4
	164	3	5.65	5.48	5.37	17.5
	165	4	5.87	5.87		23.5
	166	5	21.58	20.61		105.5
VII.	86	3	8.35	8.20		24.8
	87	3	8.41	8.68		25.6
	88	3	8.10	7.88	8.23	24.2
	89	3	19.09	19.00		57.2
	91	3	21.16	21.47	21.54	64.0
	92	3	23.21	23.09		69.4
	93	3	5.58	5.76	5.68	17.0
	94	3	9.55	9.30		28.2
	95	3	12.68	12.75		38.1
	96	3	31.69	31.65		95.0
	103	3	8.30	8.70	8.45	25.5
	104	3	0.73	0.76		2.2
	105	3	4.97	5.17		15.4
	107	3	2.91	2.88		8.6
X.	129	3	2.53	2.68	2.60	7.8
	130	3 ¹	0.58	0.58		1.7
	130	3	5.58	5.61	5.81	17.0
	131	3	0.68	0.60		1.9
	132	3	2.80	2.75		4.7
	133	3	0.51	0.62		1.7
	141	3	0.70	0.70		2.1
	143	4	0.89	0.94		3.7
	144	4	0.71	0.63	0.58	2.5
	146	4	1.15	1.15		4.6
	147	4	6.70	6.50		26.4
	149	3	0.57	0.51		1.6
	150	3	10.96	10.96		32.9
XI.	111	5 ²	18.94	18.97		105.4
	111	5	3.29	3.31		16.5
	112	5	14.73	14.73		79.2
	112	5	2.34	2.35		11.8
	112	3 ¹	6.29	6.29		18.8
	113	5 ²	4.59	4.57		27.9
	113	3 ¹	12.85	12.85		38.5
	113	5	1.57	1.50	1.66	7.9
	114	5	16.57	16.74		83.3
	114	5	7.24	7.04	{ 6.64 }	34.7
	114	5			{ 6.84 }	

¹ In residual solutions.² In solutions of 1888.

Nitrogen in Residual Solutions.—Continued.

Series No.	Experiment No.	Liters of Extract.	Nitrogen found in one liter of Extract.			Total Nitrogen in residual solution.
			A.	B.	C.	
XII.	171	4	4.39	4.46		17.7
	171	3 ¹	0.65	0.68		2.0
	172	4	2.01	2.01		8.1
	173	4	2.97	2.95		11.8
	174	4	2.27	2.27		9.1
	175	4	6.46	6.38		25.7
	177	4	10.11	10.01		40.2
	178	4	9.27	9.17		36.9
XIII.	186	6	0.69	0.72		4.1
	189	6	0.57	0.55		3.4
	191	5 ¹	21.36	21.38		106.9
	192	5	15.96	15.97		79.9
	193	6	0.80	0.79		4.8
	194	6	1.73	1.73		10.4

In the following cases, either no nitrogen was supplied in the nutritive solutions, or the residual solutions contained no nitrogen :

Series XI, Nos. 151-157, 160 and 162.

Series VII, Nos. 81-84, 97-102, 106 and 108.

Series X, Nos. 115-128, 134-140, and 145.

Series VIII, No. 109.

Series IX, Nos. 25-27, 34-36, 48-49.

Series XII, Nos. 167-170.

Series XIII, Nos. 179-185, 187, 188, 190, 195 and 196.

REVIEWS AND REPORTS.

SYNTHETICAL EXPERIMENTS IN THE SUGAR SERIES.

An interesting address¹ upon the synthesis of the sugars was given by Professor Emil Fischer at a meeting of the Berlin Chemical Society, held on June 23, 1890. On this occasion a number of new sugars and their derivatives were described for

¹ Ber. d. chem. Ges. 23, 2114.

the first time. Of especial interest is the announcement of the discovery of the optical isomers of dextrose and levulose and of gluconic and saccharic acids. So that to-day, as a result of his work, it is not only possible to prepare the natural sugars dextrose and levulose by synthetical methods, but also the optical isomers of these sugars as well. When these experiments were begun in 1887, only four sugars of the formula $C_6H_{12}O_6$ were known, namely, dextrose, levulose, galactose, and sorbinose. Now we have no less than fourteen such compounds, and all but two of them have been made synthetically. Furthermore, the existence of all these new sugars is in accordance with the deductions of the Le Bel-van't Hoff hypothesis.

Looking at Professor Fischer's work as a whole, it is worthy of note that, apart from the splendid achievement of effecting the synthesis of grape sugar and fruit sugar, he has discovered and developed several new and important reactions which are of wide application in organic chemistry. His success depends in no small degree upon the use which he has made of these reactions. Among the most important of these are undoubtedly the phenylhydrazine reactions. The hydrazones and osazones are the compounds formed by the action of phenylhydrazine upon the sugars and the compounds analogous with them. The methods for preparing these substances, as well as the more important transformations which they undergo, have already been described in previous reports¹ in this Journal. On account of the readiness with which they crystallise and the ease with which they can be obtained in pure condition they are invaluable for the purpose of detecting and purifying the sugars. The osazones, further, by treatment with hydrochloric acid are converted into osones, and these substances by reduction are transformed into ketone sugars. It is, therefore, possible in this way to change all aldehyde sugars, of which grape sugar may be taken as an example, into the corresponding ketone sugars, of which fruit sugar is a good representative. The osazones, too, are of value for determining the composition and constitution of the several sugars. The carbohydrates $C_6H_{10}O_5$, $C_6H_{12}O_6$, $C_7H_{14}O_7$, etc., all have the same percentage composition; an analysis alone, therefore, would not be sufficient for determining the molecular formula of any one of them. This result could be attained by analysing the osazone, and, moreover, the osazone possesses the advantage of being a derivative that can be easily prepared in pure condition. The hydrazones and osazones are, however, especially valuable for the isolation of new sugars, and compounds analogous to the sugars. Phenylhydrazine also unites with the acids of the sugar series and forms compounds called hydrazides. These substances have proved to be of value in separating and purifying the sugar-acids.

¹ This Journal 11, 277; 12, 357.

Another reaction of no less importance is the reduction of the lactones of the sugar-acids with nascent hydrogen. This reaction, together with the well known methods for preparing acids, make it possible to build up whole series of sugars from simpler ones containing a small number of carbon atoms. The sugars, like other aldehydes and ketones, unite directly with hydrocyanic acid, and the cyanhydrines which are thus formed can be readily transformed into acids containing one carbon atom more than the original sugar. Now by means of the reaction just mentioned it is possible to convert these acids into the corresponding sugars. Thus, for example, arabinose, $C_5H_{10}O_5$, unites with hydrocyanic acid and forms the cyanhydrine $C_5H_{11}O_5.CN$. This compound when boiled with alkalis is converted into arabinosecarbonic acid, $C_5H_{11}O_5.COOH$; and the lactone of the latter substance when reduced with sodium amalgam is transformed into *L*-mannose, $C_6H_{12}O_6$. Then the process of lengthening the chain of carbon atoms can be repeated and a sugar of the formula $C_7H_{14}O_7$ obtained. Dr. Fischer has prepared in this way from arabinose new sugars with six, seven, eight, and even nine carbon atoms. There is no reason—except perhaps lack of material—why the process should not be carried still farther.

The preparation of so many new sugars by this method has necessitated the adoption of a new nomenclature for these compounds. It is suggested¹ that a sugar be named according to the number of carbon atoms which it contains, as a triose, tetrose, pentose, hexose, heptose, octose, nonose, etc. Further, each individual isomer of the same series is to be distinguished by adding a prefix to the name which shall indicate its derivation. For example, "mannoheptose" means a sugar with seven carbon atoms, $C_7H_{14}O_7$, prepared from mannose. The names dextrose and levulose are to be dropped entirely, and instead of the former it is suggested that the name *glucose*, and for the latter *fructose* be substituted. The optical isomers are designated by means of the letters *d*, *l* and *i*, placed before the names of the sugars. The general name for a sugar containing the aldehyde group is *aldose*, and for a ketone sugar *ketose*.

The advantages of the new system of nomenclature can best be seen in the following table which contains the names of all the simple sugars known at the present time:

Triose: Glycero. (Mixture of glyceric aldehyde and dioxycetone.)

Tetrose: Erythro. (Probably a mixture of aldose and ketose.)

Pentoses: Arabinose. (Aldose.)

Xylose.

Methylpentose, or Rhamnose. (Aldose.)

¹ Ber. d. chem. Ges. 23, 2136.

Hexoses:	<i>d</i> -, <i>l</i> -, <i>i</i> -Glucose, <i>d</i> -, <i>l</i> -, <i>i</i> -Mannose, <i>d</i> -, <i>l</i> -, <i>i</i> -Fructose. (Ketose.)	} Aldose sugars, } Mannite series.
	Galactose. (Aldose of the dulcete series.)	
	Sorbinose.	} Constitution unknown.
	Formose,	
	β -Acrose,	
	Methylhexose, Rhamnohexose.	(Aldose.)
Heptoses:	Mannoheptose, Glucoheptose, Galaheptose, Fructoheptose, Methylheptose, Rhamnoheptose,	} Aldose sugars.
Octoses:	Mannoctose, Glucocose, Rhamnooctose,	
Nonnose:	Mannononose,	

Glycerose and erythrose are obtained by the oxidation of glycine and erythrite respectively. Arabinose is an aldehyde sugar with a normal chain of carbon atoms, and can be easily transformed into *l*-mannose. The constitution of xylose has not yet been determined. Rhamnose is also classified with the pentoses because it may be regarded as a methyl derivative of arabinose. Under the hexoses grape sugar is classified as *d*-glucose and fruit sugar as *d*-fructose. The optical isomers of these sugars, lately discovered, are *i*- and *l*-glucose and *i*- and *l*-fructose. Methylhexose has been prepared synthetically from rhamnose by the reactions described above. The higher sugars in the list have also been prepared by this synthetical method, and their origin is designated by the prefixes manno-, gluco-, gala-, and fructo-. Most of these new compounds surpass the derivatives of the natural hexoses in power of crystallisation. The most interesting among them is mannononose, because with yeast it ferments as easily as glucose. The octoses, heptoses and pentoses cannot be fermented, but glycerose and most of the hexoses possess this property.

Many of these new sugars will probably be found to occur in nature. Already the new sugar mannose has been found in a number of plants, and has been obtained in quantity from the reserve cellulose of vegetable ivory. There are indications that others also occur in the vegetable world. Thus mannoheptose by reduction is converted into a heptacid alcohol $C_7H_{16}O_7$ ¹, and this compound has been found to be identical with Perseite,² a compound which occurs in the fruit of *Laurus Persea*.

To show just how these new reactions were of service in effecting the synthesis of the sugars, it may, perhaps, be of interest to

¹ Ber. d. chem. Ges. 23, 2231.

² Ann. chim. phys. [6] 19, 5; This Journal 12, 284.

recall briefly the different steps in Prof. Fischer's investigation. He began his work by studying the oxidation of polyacid alcohols, such as glycerine, erythrite, and mannite. It had long been known that compounds analogous to the sugars are formed by the oxidation of these substances, but the greatest difficulty was experienced in attempting to separate these compounds from the other oxidation products. Here the phenylhydrazine reaction proved to be of great value. Glycerose and erythrose were separated in the form of beautifully crystallised osazones, the former from the oxidation products of glycerine, the latter from those of erythrite. Mannite by oxidation gave fruit sugar and a new sugar called *d*-mannose, the latter being discovered by means of its hydrazone. Mannose on investigation was found to be an aldehyde sugar of the same constitution as grape sugar, and proved to be a stereometric isomer of the latter substance. It was also found that *d*-mannonic acid by heating with quinoline could be changed into gluconic acid, and the latter compound when reduced with sodium amalgam was transformed into grape sugar. *d*-Mannose was also easily converted into fruit sugar, for on treating it with phenylhydrazine the same osazone is obtained as from grape sugar and fruit sugar, namely, glucosazone. Glucosazone by treatment with concentrated hydrochloric acid is converted into glucosone, and the latter compound by reduction is transformed into fruit sugar. It is evident, therefore, that if *d*-mannose or *d*-mannonic acid be prepared synthetically, the synthesis of dextrose and levulose is also accomplished.

The condensation of formic aldehyde and of glycerose and the action of alkalis upon acrolein bromide give rise to the formation of substances resembling the sugars. Professor Fischer and his co-workers succeeded in obtaining from these substances a sugar having the formula $C_6H_{12}O_6$, and which is known as α -acrose. The osazone of this sugar is identical with glucosazone, except that it is optically inactive. By reduction the new sugar is converted into a hexacid alcohol called α -acrite, which has all the properties of mannite but that of turning the ray of polarised light. In short α -acrite is *i*-mannite.

The next step would naturally have been to separate the α -acrite, or one of its derivatives, into the optically active components. In this way *d*- and *l*-mannite would have been obtained, and the former by easy transformations could have been converted into *d*-mannose, and this into fruit sugar; further, by oxidation the *d*-mannose could have been changed into *d*-mannonic acid, and then the latter compound could have been converted into grape sugar. These changes unfortunately could not be carried out with the α -acrite obtained by synthesis, because of lack of material. Owing to the many transformations involved in making α -acrite, and the very small yield obtained in some of the reactions, a kilogram of glycerine gave only 0.2 gram α -acrite.

The complete synthesis of the sugars would probably not have been effected so soon had not Dr. Fischer at this stage of the work made the fortunate discovery that arabinosecarbonic acid and *d*-mannonic acids are optical isomers, and that these acids combine to form an optically inactive compound of the same composition. By means of the new reaction for reducing sugar acids, this inactive acid, *i*-mannonic acid, was converted into *i*-mannose, and the latter by further reduction into *i*-mannite. But *i*-mannite and *a*-acrite are identical substances, and thus a new starting point for the synthesis of the natural sugars was obtained. *a*-Acrite, or *i*-mannite, and its derivatives, could now be prepared in quantity from arabinose and *d*-mannose, two sugars that occur in nature. It only remained to split into optically active isomers¹ *i*-mannite or one of its derivatives. The derivative selected for this purpose was *i*-mannonic acid, and this by means of its strychnine and morphine salts was biparted and *d*- and *l*-mannonic acids obtained. These bipartites by reductions were converted into *d*- and *l*-mannose, and thus all the steps in the synthesis of the sugars had been taken, for, as stated above, *d*-mannose can be transformed into fruit sugar, and *d*-mannonic acid into gluconic acid and grape sugar.

The table below (see next page) contains all the compounds belonging to the mannite group of sugars known at present, and also shows the relations which they bear to one another.

L-Gluconic acid and *L*-glucose and *L*-saccharic acids are obtained from *l*-mannonic acid in the same way that the corresponding *d* derivatives are prepared from *d*-mannonic acids. As was to be expected, these compounds resemble very closely the well known compounds of the *d* series, and each one unites with its optical isomer to form an inactive compound. In this way the three compounds *i*-gluconic acid, *i*-saccharic acid and *i*-glucose are obtained. It was also observed that *l*-gluconic acid can be obtained in abundant quantity from the mother-liquors obtained in the preparation of *l*-mannonic acid from arabinose. *L*-Glucose, like *l*-mannose and *l*-fructose, cannot be fermented with yeast, but in all other respects it is very similar to its optical isomer, except, of course, that it rotates the polarised ray as much to the left as *d*-glucose does to the right.

Omitting the compounds containing nitrogen, there are now 26 substances known that belong to the mannite series, and no compound has thus far been discovered which in any way contradicts the deductions of the Le Bel-van't Hoff hypothesis. Nevertheless, the exact relation which *d*-mannonic acid and *d*-gluconic acid

¹As there is no word in the English language for describing this kind of chemical change, it might, perhaps, be well to adopt for this purpose the following terms:

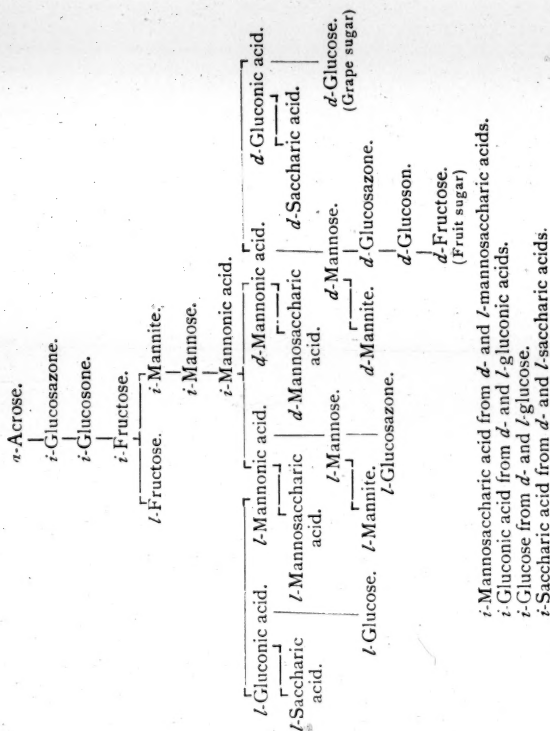
Bipart, to separate a compound into its optically active isomers (*dédoubler* in French).

Bipartition, the act of separating a compound into optically active isomers.

Bipartient, that which causes bipartition.

Bipartite, a compound that can undergo bipartition.

Bipartite, a compound obtained by bipartition.—E. H. K.



bear to each other is not yet quite clear. In general, the results of this investigation tend to confirm the principle that for every optically active compound there is an optical isomer which can unite with it to form an inactive substance. But gluconic and mannonic acids cannot be made to unite with one another to form an inactive compound, although they can be changed one into the other by heating with quinoline.

Some interesting problems for investigation suggest themselves. Grape sugar and fruit sugar appear to be the first products of

assimilation in plants; now, does the synthesis of these compounds throw any light upon their formation in nature? As yet, almost nothing is known with certainty about the method of formation of the carbohydrates in green leaves. v. Baeyer has suggested the idea that carbonic acid is first reduced to formic aldehyde, and then by condensation the latter substance is converted into sugar. But as yet no one has succeeded in obtaining evidence of the presence of at all appreciable quantities of formic aldehyde in green leaves, and, perhaps, in future investigations upon this subject better results would be obtained if the attempt were made to detect glycerose or other intermediate products by means of the new methods. Another question in physiological chemistry suggests itself. Why are only the optically active sugars of the *d*-mannite series found in nature? Are they the only products of assimilation, and is the formation of optically active substances a prerogative of living organisms, brought about by a special cause, a kind of vital force? Prof. Fischer is inclined to think that this is not the case, but that in all probability inactive substances are first formed in the plant and that these then undergo bipartition, and the sugars of the *d*-mannite series are used in the formation of starch, cellulose, inuline, etc., while the optical isomers are used for other purposes that are as yet unknown to us.

What will be the effect of the new sugars, such as mannononose and the pentoses and heptoses, upon animal bodies? What changes will these new sugars cause in the blood and tissues of animals if used as food? These are interesting problems for the physiological chemist to solve. The assimilating plant prepares from sugar not only the most complicated carbohydrates and fats, but also with the addition of nitrogenous inorganic compounds it forms proteids. Moulds and bacteria also have this power. Now, if it should be possible to nourish the plant or the bacteria with a sugar of different composition, it might be possible to obtain in this way a new kind of albumen. And with a change in the building material, may we not expect a change in the architecture? Prof. Fischer thinks that in this way it might be possible to exert a chemical influence upon organisms which would cause the most remarkable phenomena, changes of form which would far surpass anything that has hitherto been attained by breeding and intercrossing.

E. H. KEISER.

THE ACTION OF CARBON MONOXIDE ON NICKEL.

Under the above title L. Mond, C. Langer and F. Quinke have recently¹ made known the results of their investigations upon the action of carbon monoxide upon heated metals, and upon nickel in particular.

¹J. Chem. Soc. 57, 749.

"When carbon monoxide is passed over finely-divided metallic nickel at a temperature between 350° and 450° , carbon dioxide is formed, and a black amorphous powder is obtained consisting of nickel and carbon. The composition of this powder varies very widely with the temperature employed, and still more according to the time the operation has been carried on. A small amount of nickel can decompose a very large amount of carbon monoxide."

"The carbon contained in this substance is very readily attacked by steam; at the comparatively low temperature of 350° hydrogen and carbon dioxide are obtained without a trace of carbon monoxide." When the compound was heated and then allowed to cool in a current of carbon monoxide it was found that a volatile compound of nickel was obtained, for on passing the gases through a heated tube a mirror of metallic nickel was deposited on the walls of the tube. It was then found that on heating finely-divided metallic nickel to about 400° and afterwards allowing it to cool in a slow current of carbon monoxide, the gas is readily absorbed, and of the mixture of gases given off, as much as 30 per cent., in some cases, may be condensed by means of a suitable cooling apparatus and obtained in the form of a clear, volatile liquid. If the vapor of this liquid be passed through a tube heated above 150° it is decomposed with the deposition of metallic nickel, usually contaminated with carbon resulting from the action of metallic nickel upon free carbon monoxide. It was found, however, that at 180° the deposit consisted of pure nickel, and advantage was taken of this fact to make analyses of the liquid. It was found to consist of nickel and carbon monoxide in the proportions represented by the formula $\text{Ni}(\text{CO})_4$.

The vapor of "nickel-carbon-oxide," when mixed with other gases, is not affected by alkalis or acids, but it reduces a solution of copper chloride in ammonia, finally precipitating the copper as such. It also precipitates silver from ammoniacal solutions of the chloride. It is decomposed by chlorine and bromine. The electric spark slowly breaks it up into nickel and carbon monoxide. The liquid nickel-carbon-oxide is mobile, highly refractive; boils at 43° , solidifies at -25° ; its specific gravity is 1.3185 at 17° . It is unaffected by dilute acids or alkalis or by strong hydrochloric acid. The vapor density corresponds with the formula $\text{Ni}(\text{CO})_4$. Determinations of the atomic weight of nickel were made, the pure metal deposited in a heated tube being employed. The average of three results is 58.58. Numerous attempts to form similar compounds of other metals met with no success.

WYATT W. RANDALL.

THE INFLUENCE OF "NEUTRAL" SOLVENTS.

Although it has frequently been observed that the velocity of chemical action is influenced by so-called neutral solvents, only

very recently has any attempt been made systematically to study this influence. Now, however, Menshutkin, who has distinguished himself in allied fields of investigation, has taken up this subject and has determined the velocity of combination of triethylamine and ethyl iodide in twenty-three solvents.¹ His results are as follows:

1. The reaction is slowest in hydrocarbons and simple ethers; and then come the esters, the alcohols and, finally, the ketones. If we represent the coefficient of velocity in the case of hexane as 1, it is 4.2 with ethyl ether, 123.9 with ethyl acetate, 203.3 with ethyl alcohol and 337.7 with acetone.

2. When homologues are used as menstrua the velocity is greatest with that having the smallest molecular weight, and *vice versa*. With methyl alcohol it is 286.6, and with ethyl alcohol 203.3; with anisol it is 223.9, and with phenetol 117.7 (hexane = 1).

3. When two menstrua resemble each other otherwise, the velocity is greater with that having a "double bond." The benzene condition seems to favor high velocities, and so does, even more markedly, the naphthalene condition. k is equal to 203.3 with ethyl alcohol, and 240.5 with allyl alcohol. It is equal to 150 with brombenzene, and 627 with α -bromnaphthalene.

4. Chlorine derivatives give greater values for k than the hydrocarbons themselves, and smaller values than bromine derivatives. The value of k with benzene being 38.2, it is 128 with chlorbenzene, and 150 with brombenzene.

These results show that the influence of solvents on the velocity of chemical action depends upon their chemical and not upon their physical properties, and that similar effects are produced by similar changes in the menstrua and in the reagents. An illustration will make this clear. The velocity is greater if methyl alcohol is used as a solvent than if ethyl alcohol is so employed, and it is greater with methyl iodide as a reagent than with ethyl iodide. This similarity seems to show that solution in organic solvents is not purely mechanical, but is, perhaps, as complicated as solution in water, and strengthens the view that solution is but the first phase of what in its later phases is known as chemical action.

F. LENGFELD.

¹ Ztschr. phys. Chem. 6, 41.